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(54) Title: PRODUCTION OF GAMMA LINOLENIC ACID BY A Δ6-DESATURASE

(57) Abstract

Linoleic acid is converted into γ-linolenic acid by the enzyme Δ6-desaturase. The present invention is directed to isolated nucleic acids comprising the Δ6-desaturase gene. More particularly, the isolated nucleic acid comprises the promoter, coding region and termination regions of the Δ6-desaturase gene. The present invention provides recombinant constructions comprising the Δ6-desaturase coding region in functional combination with heterologous regulatory sequences. The nucleic acids and recombinant constructions of the instant invention are useful in the production of GLA in transgenic organisms.

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1   ## PRODUCTION OF GAMMA LINOLENIC ACID BY A Δ6-DESATURASE

Linoleic acid (18:2) (LA) is transformed
into gamma linolenic acid (18:3) (GLA) by the enzyme
5   Δ6-desaturase. When this enzyme, or the nucleic acid
encoding it, is transferred into LA-producing cells,
GLA is produced. The present invention provides
nucleic acids comprising the Δ6-desaturase gene. More
specifically, the nucleic acids comprise the
10   promoters, coding regions and termination regions of
the Δ6-desaturase genes. The present invention is
further directed to recombinant constructions
comprising a Δ6-desaturase coding region in functional
combination with heterologous regulatory sequences.
15   The nucleic acids and recombinant constructions of the
instant invention are useful in the production of GLA
in transgenic organisms.

Unsaturated fatty acids such as linoleic
($C_{18}\Delta^{9,12}$) and α-linolenic ($C_{18}\Delta^{9,12,15}$) acids are essential
20   dietary constituents that cannot be synthesized by
vertebrates since vertebrate cells can introduce
double bonds at the Δ^9 position of fatty acids but
cannot introduce additional double bonds between the
Δ^9 double bond and the methyl-terminus of the fatty
25   acid chain. Because they are precursors of other
products, linoleic and α-linolenic acids are essential
fatty acids, and are usually obtained from plant
sources. Linoleic acid can be converted by mammals
into γ-linolenic acid (GLA, $C_{18}\Delta^{6,9,12}$) which can in turn
30   be converted to arachidonic acid (20:4), a critically

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1   important fatty acid since it is an essential
     precursor of most prostaglandins.
             The dietary provision of linoleic acid, by
     virtue of its resulting conversion to GLA and
5   arachidonic acid, satisfies the dietary need for GLA
     and arachidonic acid. However, a relationship has
     been demonstrated between consumption of saturated
     fats and health risks such as hypercholesterolemia,
     atherosclerosis and other clinical disorders which
10   correlate with susceptibility to coronary disease,
     while the consumption of unsaturated fats has been
     associated with decreased blood cholesterol
     concentration and reduced risk of atherosclerosis.
     The therapeutic benefits of dietary GLA may result
15   from GLA being a precursor to arachidonic acid and
     thus subsequently contributing to prostaglandin
     synthesis. Accordingly, consumption of the more
     unsaturated GLA, rather than linoleic acid, has
     potential health benefits. However, GLA is not
20   present in virtually any commercially grown crop
     plant.
             Linoleic acid is converted into GLA by the
     enzyme Δ6-desaturase. Δ6-desaturase, an enzyme of
     more than 350 amino acids, has a membrane-bound domain
25   and an active site for desaturation of fatty acids.
     When this enzyme is transferred into cells which
     endogenously produce linoleic acid but not GLA, GLA is
     produced. The present invention, by providing the
     gene encoding Δ6-desaturase, allows the production of
30   transgenic organisms which contain functional Δ6-
     desaturase and which produce GLA. In addition to

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1   allowing production of large amounts of GLA, the
    present invention provides new dietary sources of GLA.
            The present invention is directed to
    isolated Δ6-desaturase genes. Specifically, the
5   isolated genes comprises the Δ6-desaturase promoters,
    coding regions, and termination regions.
            The present invention is further directed to
    expression vectors comprising the Δ6-desaturase
    promoter, coding region and termination region.
10   Yet another aspect of this invention is
    directed to expression vectors comprising a Δ6-
    desaturase coding region in functional combination
    with heterologous regulatory regions, i.e. elements
    not derived from the Δ6-desaturase gene.
15   Cells and organisms comprising the vectors
    of the present invention, and progeny of such
    organisms, are also provided by the present invention.
            A further aspect of the present invention
    provides isolated bacterial Δ6-desaturase. An
20 isolated plant Δ6-desaturase is also provided.
            Yet another aspect of this invention
    provides a method for producing plants with increased
    gamma linolenic acid content.
            A method for producing chilling tolerant
25 plants is also provided by the present invention.
            Fig. 1 depicts the hydropathy profiles of
    the deduced amino acid sequences of Synechocystis Δ6-
    desaturase (Panel A) and Δ12-desaturase (Panel B).
    Putative membrane spanning regions are indicated by
30 solid bars. Hydrophobic index was calculated for a

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1   window size of 19 amino acid residues [Kyte, et al.
    (1982) J. Molec. Biol. 157].
                Fig. 2 provides gas liquid chromatography
    profiles of wild type (Panel A) and transgenic (Panel
5   B) Anabaena.
                Fig. 3 is a diagram of maps of cosmid cSy75,
    cSy13 and Csy7 with overlapping regions and subclones.
    The origins of subclones of Csy75, Csy75-3.5 and Csy7
    are indicated by the dashed diagonal lines.
10 Restriction sites that have been inactivated are in
    parentheses.
                Fig. 4 provides gas liquid chromatography
    profiles of wild type (Panel A) and transgenic (Panel
    B) tobacco.
15   Fig. 5A depicts the DNA sequence of a Δ-6
    desaturase cDNA isolated from borage.
                Fig. 5B depicts the protein sequence of the
    open reading frame in the isolated borage Δ-6
    desaturase cDNA. Three amino acid motifs
20 characteristic of desaturases are indicated and are,
    in order, lipid box, metal box 1, and metal box 2.
                Fig. 6 is a dendrogram showing similarity of
    the borage Δ6-desaturase to other membrane-bound
    desaturases. The amino acid sequence of the borage
25 Δ6-desaturase was compared to other known desaturases
    using Gene Works (IntelliGenetics). Numerical values
    correlate to relative phylogenetic distances between
    subgroups compared.
                Fig. 7 is a restriction map of 221.Δ6.NOS
30 and 121.Δ6.NOS. In 221.Δ6.NOS, the remaining portion

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1   of the plasmid is pBI221 and in 121.Δ6.NOS, the
    remaining portion of the plasmid is pBI121.

         Fig. 8 provides gas liquid chromatography
    profiles of mock transfected (Panel A) and 221.Δ6.NOS

5   transfected (Panel B) carrot cells. The positions of
    18:2, 18:3 α, and 18:3 γ(GLA) are indicated.

         Fig. 9 provides gas liquid chromatography
    profiles of an untransformed tobacco leaf (Panel A)
    and a tobacco leaf transformed with 121.Δ6.NOS. The

10 positions of 18:2, 18:3 α, 18:3γ(GLA), and 18:4 are
    indicated.

         Fig. 10 provides gas liquid chromotography
    profiles for untransformed tobacco seeds (Panel A) and
    seeds of tobacco transformed with 121.Δ6.NOS. The

15 positions of 18:2, 18:3α and 18:3γ(GLA) are indicated.

         The present invention provides isolated
    nucleic acids encoding Δ6-desaturase. To identify a
    nucleic acid encoding Δ6-desaturase, DNA is isolated
    from an organism which produces GLA. Said organism

20 can be, for example, an animal cell, certain fungi
    (e.g. Mortierella), certain bacteria (e.g.
    Synechocystis) or certain plants (borage, Oenothera,
    currants). The isolation of genomic DNA can be
    accomplished by a variety of methods well-known to one

25 of ordinary skill in the art, as exemplified by
    Sambrook et al. (1989) in Molecular Cloning: A
    Laboratory Manual, Cold Spring Harbor, NY. The
    isolated DNA is fragmented by physical methods or
    enzymatic digestion and cloned into an appropriate

30 vector, e.g. a bacteriophage or cosmid vector, by any
    of a variety of well-known methods which can be found

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1   in references such as Sambrook et al. (1989).
    Expression vectors containing the DNA of the present
    invention are specifically contemplated herein. DNA
    encoding Δ6-desaturase can be identified by gain of
5   function analysis. The vector containing fragmented
    DNA is transferred, for example by infection,
    transconjugation, transfection, into a host organism
    that produces linoleic acid but not GLA. As used
    herein, "transformation" refers generally to the
10 incorporation of foreign DNA into a host cell.
    Methods for introducing recombinant DNA into a host
    organism are known to one of ordinary skill in the art
    and can be found, for example, in Sambrook et al.
    (1989). Production of GLA by these organisms (i.e.,
15 gain of function) is assayed, for example by gas
    chromatography or other methods known to the
    ordinarily skilled artisan. Organisms which are
    induced to produce GLA, i.e. have gained function by
    the introduction of the vector, are identified as
20 expressing DNA encoding Δ6-desaturase, and said DNA is
    recovered from the organisms. The recovered DNA can
    again be fragmented, cloned with expression vectors,
    and functionally assessed by the above procedures to
    define with more particularity the DNA encoding Δ6-
25 desaturase.
            As an example of the present invention,
    random DNA is isolated from the cyanobacteria
    Synechocystis Pasteur Culture Collection (PCC) 6803,
    American Type Culture Collection (ATCC) 27184, cloned
30 into a cosmid vector, and introduced by
    transconjugation into the GLA-deficient cyanobacterium

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1   <u>Anabaena</u> strain PCC 7120, ATCC 27893. Production of
    GLA from <u>Anabaena</u> linoleic acid is monitored by gas
    chromatography and the corresponding DNA fragment is
    isolated.

5   The isolated DNA is sequenced by methods
    well-known to one of ordinary skill in the art as
    found, for example, in Sambrook <u>et</u> <u>al</u>. (1989).
         In accordance with the present invention,
    DNA molecules comprising Δ6-desaturase genes have been
10 isolated. More particularly, a 3.588 kilobase (kb)
    DNA comprising a Δ6-desaturase gene has been isolated
    from the cyanobacteria <u>Synechocystis</u>. The nucleotide
    sequence of the 3.588 kb DNA was determined and is
    shown in SEQ ID NO:1. Open reading frames defining
15 potential coding regions are present from nucleotide
    317 to 1507 and from nucleotide 2002 to 3081. To
    define the nucleotides responsible for encoding Δ6-
    desaturase, the 3.588 kb fragment that confers Δ6-
    desaturase activity is cleaved into two subfragments,
20 each of which contains only one open reading frame.
    Fragment ORF1 contains nucleotides 1 through 1704,
    while fragment ORF2 contains nucleotides 1705 through
    3588. Each fragment is subcloned in both forward and
    reverse orientations into a conjugal expression vector
25 (AM542, Wolk <u>et</u> <u>al</u>. [1984] <u>Proc. Natl. Acad. Sci. USA</u>
    <u>81</u>, 1561) that contains a cyanobacterial carboxylase
    promoter. The resulting constructs (i.e. ORF1(F),
    ORF1(R), ORF2(F) and ORF2(R)] are conjugated to wild-
    type <u>Anabaena</u> PCC 7120 by standard methods (see, for
30 example, Wolk et al. (1984) <u>Proc. Natl. Acad. Sci. USA</u>
    <u>81</u>, 1561). Conjugated cells of <u>Anabaena</u> are

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1   identified as Neo^R green colonies on a brown
    background of dying non-conjugated cells after two
    weeks of growth on selective media (standard mineral
    media BG11N + containing 30μg/ml of neomycin according
5   to Rippka et al., (1979) <u>J. Gen Microbiol. 111</u>, 1).
    The green colonies are selected and grown in selective
    liquid media (BG11N + with 15μg/ml neomycin). Lipids
    are extracted by standard methods (e.g. Dahmer et al.,
    (1989) <u>Journal of American Oil Chemical Society 66</u>,
10 543) from the resulting transconjugants containing the
    forward and reverse oriented ORF1 and ORF2 constructs.
    For comparison, lipids are also extracted from wild-
    type cultures of <u>Anabaena</u> and <u>Synechocystis</u>. The
    fatty acid methyl esters are analyzed by gas liquid
15 chromatography (GLC), for example with a Tracor-560
    gas liquid chromatograph equipped with a hydrogen
    flame ionization detector and a capillary column. The
    results of GLC analysis are shown in Table 1.

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1 Table 1:   Occurrence of C18 fatty acids in wild-type
   and   transgenic cyanobacteria

SOURCE	18:0	18:1	18:2	γ18:3	α18:3	18:4
Anabaena (wild type)	+	+	+	-	+	-
Anabaena + ORF1(F)	+	+	+	-	+	-
Anabaena + ORF1(R)	+	+	+	-	+	-
Anabaena + ORF2(F)	+	+	+	+	+	+
Anabaena + ORF2(R)	+	+	+	-	+	-
Synechocystis (wild type)	+	+	+	+	-	-

As assessed by GLC analysis, GLA deficient

15 _Anabaena_ gain the function of GLA production when the
construct containing ORF2 in forward orientation is
introduced by transconjugation. Transconjugants
containing constructs with ORF2 in reverse orientation
to the carboxylase promoter, or ORF1 in either

20 orientation, show no GLA production. This analysis
demonstrates that the single open reading frame (ORF2)
within the 1884 bp fragment encodes ∆6-desaturase.
The 1884 bp fragment is shown as SEQ ID NO:3. This is
substantiated by the overall similarity of the

25 hydropathy profiles between ∆6-desaturase and ∆12-
desaturase [Wada _et al_. (1990) _Nature_ 347] as shown in
Fig. 1 as (A) and (B), respectively.

       Also in accordance with the present
invention, a cDNA comprising a ∆6-desaturase gene from
borage (_Borago officinalis_) has been isolated. The

30 nucleotide sequence of the 1.685 kilobase (kb) cDNA

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1 was determined and is shown in Fig. 5A (SEQ ID NO: 4).
   The ATG start codon and stop codon are underlined.
   The amino acid sequence corresponding to the open
   reading frame in the borage delta 6-desaturase is
5 shown in Fig. 5B (SEQ ID NO: 5).
           Isolated nucleic acids encoding Δ6-
   desaturase can be identified from other GLA-producing
   organisms by the gain of function analysis described
   above, or by nucleic acid hybridization techniques
10 using the isolated nucleic acid which encodes
   Synechocystis or borage Δ6-desaturase as a
   hybridization probe. Both genomic and cDNA cloning
   methods are known to the skilled artisan and are
   contemplated by the present invention. The
15 hybridization probe can comprise the entire DNA
   sequence disclosed as SEQ. ID NO:1 or SEQ. ID NO:4, or
   a restriction fragment or other DNA fragment thereof,
   including an oligonucleotide probe. Methods for
   cloning homologous genes by cross-hybridization are
20 known to the ordinarily skilled artisan and can be
   found, for example, in Sambrook (1989) and Beltz et
   al. (1983) Methods in Enzymology 100, 266.
           In another method of identifying a delta 6-
   desaturase gene from an organism producing GLA, a cDNA
25 library is made from poly-A⁺ RNA isolated from
   polysomal RNA. In order to eliminate hyper-abundant
   expressed genes from the cDNA population, cDNAs or
   fragments thereof corresponding to hyper-abundant
   cDNAs genes are used as hybridization probes to the
30 cDNA library. Non hybridizing plaques are excised and
   the resulting bacterial colonies are used to inoculate

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1 liquid cultures and sequenced. For example, as a
   means of eliminating other seed storage protein cDNAs
   from a cDNA library made from borage polysomal RNA,
   cDNAs corresponding to abundantly expressed seed
5 storage proteins are first hybridized to the cDNA
   library. The "subtracted" DNA library is then used to
   generate expressed sequence tags (ETSs) and such tags
   are used to scan a data base such as GenBank to
   identify potential desaturates.

10   Transgenic organisms which gain the function
   of GLA production by introduction of DNA encoding Δ-
   desaturase also gain the function of
   octadecatetraeonic acid ($18:4^{\Delta 6,9,12,15}$) production.
   Octadecatetraeonic acid is present normally in fish
15 oils and in some plant species of the Boraginaceae
   family (Craig et al. [1964] J. Amer. Oil Chem. Soc.
   41, 209-211; Gross et al. [1976] Can. J. Plant Sci.
   56, 659-664). In the transgenic organisms of the
   present invention, octadecatetraenoic acid results
20 from further desaturation of α-linolenic acid by Δ6-
   desaturase or desaturation of GLA by Δ15-desaturase.
          The 359 amino acids encoded by ORF2, i.e.
   the open reading frame encoding Synechocystis Δ6-
   desaturase, are shown as SEQ. ID NO:2. The open
25 reading frame encoding the borage Δ6-desaturase is
   shown in SEQ ID NO: 5. The present invention further
   contemplates other nucleotide sequences which encode
   the amino acids of SEQ ID NO:2 and SEQ ID NO: 5. It
   is within the ken of the ordinarily skilled artisan to
30 identify such sequences which result, for example,
   from the degeneracy of the genetic code. Furthermore,

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1   one of ordinary skill in the art can determine, by the
     gain of function analysis described hereinabove,
     smaller subfragments of the fragments containing the
     open reading frames which encode Δ6-desaturases.

5   The present invention contemplates any such
     polypeptide fragment of Δ6-desaturase and the nucleic
     acids therefor which retain activity for converting LA
     to GLA.

            In another aspect of the present invention,
10   a vector containing a nucleic acid of the present
     invention or a smaller fragment containing the
     promoter, coding sequence and termination region of a
     Δ6-desaturase gene is transferred into an organism,
     for example, cyanobacteria, in which the Δ6-desaturase

15   promoter and termination regions are functional.
     Accordingly, organisms producing recombinant Δ6-
     desaturase are provided by this invention. Yet
     another aspect of this invention provides isolated Δ6-
     desaturase, which can be purified from the recombinant

20   organisms by standard methods of protein purification.
     (For example, see Ausubel et al. [1987] Current
     Protocols in Molecular Biology, Green Publishing
     Associates, New York).

            Vectors containing DNA encoding Δ6-
25   desaturase are also provided by the present invention.
     It will be apparent to one of ordinary skill in the
     art that appropriate vectors can be constructed to
     direct the expression of the Δ6-desaturase coding
     sequence in a variety of organisms. Replicable

30   expression vectors are particularly preferred.
     Replicable expression vectors as described herein are

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1   DNA or RNA molecules engineered for controlled
    expression of a desired gene, i.e. the Δ6-desaturase
    gene. Preferably the vectors are plasmids,
    bacteriophages, cosmids or viruses. Shuttle vectors,
5   e.g. as described by Wolk et al. (1984) Proc. Natl.
    Acad. Sci. USA, 1561-1565 and Bustos et al. (1991) J.
    Bacteriol. 174, 7525-7533, are also contemplated in
    accordance with the present invention. Sambrook et
    al. (1989), Goeddel, ed. (1990) Methods in Enzymology
10 185 Academic Press, and Perbal (1988) A Practical
    Guide to Molecular Cloning, John Wiley and Sons, Inc.,
    provide detailed reviews of vectors into which a
    nucleic acid encoding the present Δ6-desaturase can be
    inserted and expressed. Such vectors also contain
15 nucleic acid sequences which can effect expression of
    nucleic acids encoding Δ6-desaturase. Sequence
    elements capable of effecting expression of a gene
    product include promoters, enhancer elements, upstream
    activating sequences, transcription termination
20 signals and polyadenylation sites. Both constitutive
    and tissue specific promoters are contemplated. For
    transformation of plant cells, the cauliflower mosaic
    virus (CaMV) 35S promoter and promoters which are
    regulated during plant seed maturation are of
25 particular interest. All such promoter and
    transcriptional regulatory elements, singly or in
    combination, are contemplated for use in the present
    replicable expression vectors and are known to one of
    ordinary skill in the art. The CaMV 35S promoter is
30 described, for example, by Restrepo et al. (1990)

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1   <u>Plant Cell</u> <u>2</u>, 987. Genetically engineered and mutated
     regulatory sequences are also contemplated.

            The ordinarily skilled artisan can determine
     vectors and regulatory elements suitable for

5   expression in a particular host cell. For example, a
     vector comprising the promoter from the gene encoding
     the carboxylase of <u>Anabaena</u> operably linked to the
     coding region of Δ6-desaturase and further operably
     linked to a termination signal from <u>Synechocystis</u> is

10   appropriate for expression of Δ6-desaturase in
     cyanobacteria. "Operably linked" in this context
     means that the promoter and terminator sequences
     effectively function to regulate transcription. As a
     further example, a vector appropriate for expression

15   of Δ6-desaturase in transgenic plants can comprise a
     seed-specific promoter sequence derived from
     helianthinin, napin, or glycinin operably linked to
     the Δ6-desaturase coding region and further operably
     linked to a seed termination signal or the nopaline

20   synthase termination signal. As a still further
     example, a vector for use in expression of Δ 6-
     desaturase in plants can comprise a constitutive
     promoter or a tissue specific promoter operably linked
     to the Δ 6-desaturase coding region and further

25   operably linked to a constitutive or tissue specific
     terminator or the nopaline synthase termination
     signal.

            In particular, the helianthinin regulatory
     elements disclosed in applicant's copending U.S.

30   Application Serial No. 682,354, filed April 8, 1991
     and incorporated herein by reference, are contemplated

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1   as promoter elements to direct the expression of the
    Δ6-desaturase of the present invention.

            Modifications of the nucleotide sequences or
    regulatory elements disclosed herein which maintain
5   the functions contemplated herein are within the scope
    of this invention. Such modifications include
    insertions, substitutions and deletions, and
    specifically substitutions which reflect the
    degeneracy of the genetic code.

10   Standard techniques for the construction of
    such hybrid vectors are well-known to those of
    ordinary skill in the art and can be found in
    references such as Sambrook et al. (1989), or any of
    the myriad of laboratory manuals on recombinant DNA
15 technology that are widely available. A variety of
    strategies are available for ligating fragments of
    DNA, the choice of which depends on the nature of the
    termini of the DNA fragments. It is further
    contemplated in accordance with the present invention
20 to include in the hybrid vectors other nucleotide
    sequence elements which facilitate cloning, expression
    or processing, for example sequences encoding signal
    peptides, a sequence encoding KDEL, which is required
    for retention of proteins in the endoplasmic reticulum
25 or sequences encoding transit peptides which direct
    Δ6-desaturase to the chloroplast. Such sequences are
    known to one of ordinary skill in the art. An
    optimized transit peptide is described, for example,
    by Van den Broeck et al. (1985) Nature 313, 358.
30 Prokaryotic and eukaryotic signal sequences are

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1   disclosed, for example, by Michaelis et al. (1982)
     Ann. Rev. Microbiol. 36, 425.
          A further aspect of the instant invention
     provides organisms other than cyanobacteria or plants
5   which contain the DNA encoding the Δ6-desaturase of
     the present invention. The transgenic organisms
     contemplated in accordance with the present invention
     include bacteria, cyanobacteria, fungi, and plants and
     animals. The isolated DNA of the present invention
10   can be introduced into the host by methods known in
     the art, for example infection, transfection,
     transformation or transconjugation. Techniques for
     transferring the DNA of the present invention into
     such organisms are widely known and provided in
15   references such as Sambrook et al. (1989).
          A variety of plant transformation methods
     are known. The Δ6-desaturase gene can be introduced
     into plants by a leaf disk transformation-regeneration
     procedure as described by Horsch et al. (1985) Science
20   227, 1229. Other methods of transformation, such as
     protoplast culture (Horsch et al. (1984) Science 223,
     496; DeBlock et al. (1984) EMBO J. 2, 2143; Barton et
     al. (1983) Cell 32, 1033) can also be used and are
     within the scope of this invention. In a preferred
25   embodiment plants are transformed with Agrobacterium-
     derived vectors. However, other methods are available
     to insert the Δ6-desaturase genes of the present
     invention into plant cells. Such alternative methods
     include biolistic approaches (Klein et al. (1987)
30   Nature 327, 70), electroporation, chemically-induced
     DNA uptake, and use of viruses or pollen as vectors.

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1   When necessary for the transformation
method, the Δ6-desaturase genes of the present
invention can be inserted into a plant transformation
vector, e.g. the binary vector described by Bevan
5   (1984) Nucleic Acids Res. 12, 8111. Plant
transformation vectors can be derived by modifying the
natural gene transfer system of Agrobacterium
tumefaciens. The natural system comprises large Ti
(tumor-inducing)-plasmids containing a large segment,
10 known as T-DNA, which is transferred to transformed
plants. Another segment of the Ti plasmid, the vir
region, is responsible for T-DNA transfer. The T-DNA
region is bordered by terminal repeats. In the
modified binary vectors the tumor-inducing genes have
15 been deleted and the functions of the vir region are
utilized to transfer foreign DNA bordered by the T-DNA
border sequences. The T-region also contains a
selectable marker for antibiotic resistance, and a
multiple cloning site for inserting sequences for
20 transfer. Such engineered strains are known as
"disarmed" A. tumefaciens strains, and allow the
efficient transformation of sequences bordered by the
T-region into the nuclear genomes of plants.
        Surface-sterilized leaf disks are inoculated
25 with the "disarmed" foreign DNA-containing A.
tumefaciens, cultured for two days, and then
transferred to antibiotic-containing medium.
Transformed shoots are selected after rooting in
medium containing the appropriate antibiotic,
30 transferred to soil and regenerated.

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1   Another aspect of the present invention
provides transgenic plants or progeny of these plants
containing the isolated DNA of the invention. Both
monocotyledenous and dicotyledenous plants are

5   contemplated. Plant cells are transformed with the
isolated DNA encoding Δ6-desaturase by any of the
plant transformation methods described above. The
transformed plant cell, usually in a callus culture or
leaf disk, is regenerated into a complete transgenic

10   plant by methods well-known to one of ordinary skill
in the art (e.g. Horsch et al. (1985) Science 227,
1129). In a preferred embodiment, the transgenic
plant is sunflower, oil seed rape, maize, tobacco,
peanut or soybean. Since progeny of transformed

15   plants inherit the DNA encoding Δ6-desaturase, seeds
or cuttings from transformed plants are used to
maintain the transgenic plant line.
           The present invention further provides a
method for providing transgenic plants with an

20   increased content of GLA. This method includes
introducing DNA encoding Δ6-desaturase into plant
cells which lack or have low levels of GLA but contain
LA, and regenerating plants with increased GLA content
from the transgenic cells. In particular,

25   commercially grown crop plants are contemplated as the
transgenic organism, including, but not limited to,
sunflower, soybean, oil seed rape, maize, peanut and
tobacco.
           The present invention further provides a

30   method for providing transgenic organisms which
contain GLA. This method comprises introducing DNA

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1   encoding ᴧ6-desaturase into an organism which lacks or
     has low levels of GLA, but contains LA. In another
     embodiment, the method comprises introducing one or
     more expression vectors which comprise DNA encoding
5   ᴧ12-desaturase and ᴧ6-desaturase into organisms which
     are deficient in both GLA and LA. Accordingly,
     organisms deficient in both LA and GLA are induced to
     produce LA by the expression of ᴧ12-desaturase, and
     GLA is then generated due to the expression of ᴧ6-
10   desaturase. Expression vectors comprising DNA
     encoding ᴧ12-desaturase, or ᴧ12-desaturase and ᴧ6-
     desaturase, can be constructed by methods of
     recombinant technology known to one of ordinary skill
     in the art (Sambrook et al., 1989) and the published
15   sequence of ᴧ12-desaturase (Wada et al [1990] Nature
     (London) 347, 200-203. In addition, it has been
     discovered in accordance with the present invention
     that nucleotides 2002-3081 of SEQ. ID NO:1 encode
     cyanobacterial ᴧ12-desaturase. Accordingly, this
20   sequence can be used to construct the subject
     expression vectors. In particular, commercially grown
     crop plants are contemplated as the transgenic
     organism, including, but not limited to, sunflower,
     soybean, oil seed rape, maize, peanut and tobacco.
25   The present invention is further directed to
     a method of inducing chilling tolerance in plants.
     Chilling sensitivity may be due to phase transition of
     lipids in cell membranes. Phase transition
     temperature depends upon the degree of unsaturation of
30   fatty acids in membrane lipids, and thus increasing
     the degree of unsaturation, for example by introducing

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1 ⊿6-desaturase to convert LA to GLA, can induce or
improve chilling resistance. Accordingly, the present
method comprises introducing DNA encoding ⊿6-
desaturase into a plant cell, and regenerating a plant
5 with improved chilling resistance from said
transformed plant cell. In a preferred embodiment,
the plant is a sunflower, soybean, oil seed rape,
maize, peanut or tobacco plant.

The following examples further illustrate
10 the present invention.

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1   **EXAMPLE 1**
                Strains and Culture Conditions

             <u>Synechocystis</u> (PCC 6803, ATCC 27184),
5   <u>Anabaena</u> (PCC 7120, ATCC 27893) and <u>Synechococcus</u> (PCC
     7942, ATCC 33912) were grown photoautotrophically at
     30°C in BG11N+ medium (Rippka <u>et al</u>. [1979] <u>J. Gen.</u>
     <u>Microbiol. 111</u>, 1-61) under illumination of
     incandescent lamps
10   $(60\mu E.m^{-2}.S^{-1})$. Cosmids and plasmids were selected and
     propagated in <u>Escherichia coli</u> strain DH5α on LB
     medium supplemented with antibiotics at standard
     concentrations as described by Maniatis <u>et al</u>. (1982)
     <u>Molecular Cloning: A Laboratory Manual</u>, Cold Spring
15   Harbor Laboratory, Cold Spring, New York.

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1   **EXAMPLE 2**
        Construction of <u>Synechocystis</u> Cosmid Genomic Library

                Total genomic DNA from <u>Synechocystis</u> (PCC
5   6803) was partially digested with <u>Sau</u>3A and
    fractionated on a sucrose gradient (Ausubel <u>et</u> <u>al</u>.
    [1987] <u>Current Protocols in Molecular Biology</u>, Greene
    Publishing Associates and Wiley Interscience, New
    York). Fractions containing 30 to 40 kb DNA fragments
10 were selected and ligated into the dephosphorylated
    <u>Bam</u>HI site of the cosmid vector, pDUCA7 (Buikema <u>et</u>
    <u>al</u>. [1991] <u>J. Bacteriol.</u> <u>173</u>, 1879-1885). The ligated
    DNA was packaged <u>in</u> <u>vitro</u> as described by Ausubel <u>et</u>
    <u>al</u>. (1987), and packaged phage were propagated in <u>E.</u>
15 <u>coli</u> DH5α containing the <u>Ava</u>I and <u>Eco</u>4711 methylase
    helper plasmid, pRL528 as described by Buikema <u>et</u> <u>al</u>.
    (1991). A total of 1152 colonies were isolated
    randomly and maintained individually in twelve 96-well
    microtiter plates.

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1   **EXAMPLE 3**
                Gain-of-Function Expression of GLA in Anabaena

                 Anabaena (PCC 7120), a filamentous
5   cyanobacterium, is deficient in GLA but contains
     significant amounts of linoleic acid, the precursor
     for GLA (Figure 2; Table 2). The Synechocystis cosmid
     library described in Example 2 was conjugated into
     Anabaena (PCC 7120) to identify transconjugants that
10   produce GLA. Anabaena cells were grown to mid-log
     phase in BG11N+ liquid medium and resuspended in the
     same medium to a final concentration of approximately
     $2x10^3$ cells per ml. A mid-log phase culture of E.
     coli RP4 (Burkardt et al. [1979] J. Gen. Microbiol.
15   114, 341-348) grown in LB containing ampicillin was
     washed and resuspended in fresh LB medium. Anabaena
     and RP4 were then mixed and spread evenly on BG11N+
     plates containing 5% LB. The cosmid genomic library
     was replica plated onto LB plates containing 50 µg/ml
20   kanamycin and 17.5 µg/ml chloramphenicol and was
     subsequently patched onto BG11N+ plates containing
     Anabaena and RP4. After 24 hours of incubation at
     30°C, 30 µg/ml of neomycin was underlaid; and
     incubation at 30°C was continued until transconjugants
25   appeared.
                 Individual transconjugants were isolated
     after conjugation and grown in 2 ml BG11N+ liquid
     medium with 15 µg/ml neomycin. Fatty acid methyl
     esters were prepared from wild type cultures and
30   cultures containing pools of ten transconjugants as
     follows. Wild type and transgenic cyanobacterial

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1   cultures were harvested by centrifugation and washed
    twice with distilled water. Fatty acid methyl esters
    were extracted from these cultures as described by
    Dahmer et al. (1989) J. Amer. Oil. Chem. Soc. 66, 543-
5   548 and were analyzed by Gas Liquid Chromatography
    (GLC) using a Tracor-560 equipped with a hydrogen
    flame ionization detector and capillary column (30 m x
    0.25 mm bonded FSOT Superox II, Alltech Associates
    Inc., IL). Retention times and co-chromatography of
10 standards (obtained from Sigma Chemical Co.) were used
    for identification of fatty acids. The average fatty
    acid composition was determined as the ratio of peak
    area of each C18 fatty acid normalized to an internal
    standard.

15   Representative GLC profiles are shown in
    Fig. 2. C18 fatty acid methyl esters are shown.
    Peaks were identified by comparing the elution times
    with known standards of fatty acid methyl esters and
    were confirmed by gas chromatography-mass
20 spectrometry. Panel A depicts GLC analysis of fatty
    acids of wild type Anabaena. The arrow indicates the
    migration time of GLA. Panel B is a GLC profile of
    fatty acids of transconjugants of Anabaena with
    pAM542+1.8F. Two GLA producing pools (of 25 pools
25 representing 250 transconjugants) were identified that
    produced GLA. Individual transconjugants of each GLA
    positive pool were analyzed for GLA production; two
    independent transconjugants, AS13 and AS75, one from
    each pool, were identified which expressed significant
30 levels of GLA and which contained cosmids, cSy13 and
    cSy75, respectively (Figure 3). The cosmids overlap

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1   in a region approximately 7.5 kb in length. A 3.5 kb
    NheI fragment of cSy75 was recloned in the vector
    pDUCA7 and transferred to Anabaena resulting in gain-
    of-function expression of GLA (Table 2).

5   Two NheI/Hind III subfragments (1.8 and 1.7
    kb) of the 3.5 kb Nhe I fragment of cSy75-3.5 were
    subcloned into "pBLUESCRIPT" (Stratagene) (Figure 3)
    for sequencing. Standard molecular biology techniques
    were performed as described by Maniatis et al. (1982)

10 and Ausubel et al. (1987). Dideoxy sequencing (Sanger
    et al. [1977] Proc. Natl. Acad. Sci. USA 74, 5463-
    5467) of pBS1.8 was performed with "SEQUENASE" (United
    States Biochemical) on both strands by using specific
    oligonucleotide primers synthesized by the Advanced

15 DNA Technologies Laboratory (Biology Department, Texas
    A & M University). DNA sequence analysis was done
    with the GCG (Madison, WI) software as described by
    Devereux et al. (1984) Nucleic Acids Res. 12, 387-395.
             Both NheI/HindIII subfragments were

20 transferred into a conjugal expression vector, AM542,
    in both forward and reverse orientations with respect
    to a cyanobacterial carboxylase promoter and were
    introduced into Anabaena by conjugation.
    Transconjugants containing the 1.8 kb fragment in the

25 forward orientation (AM542-1.8F) produced significant
    quantities of GLA and octadecatetraenoic acid (Figure
    2; Table 2). Transconjugants containing other
    constructs, either reverse oriented 1.8 kb fragment or
    forward and reverse oriented 1.7 kb fragment, did not

30 produce detectable levels of GLA (Table 2).

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1   Figure 2 compares the C18 fatty acid profile
    of an extract from wild type Anabaena (Figure 2A) with
    that of transgenic Anabaena containing the 1.8 kb
    fragment of cSy75-3.5 in the forward orientation
5   (Figure 2B). GLC analysis of fatty acid methyl esters
    from AM542-1.8F revealed a peak with a retention time
    identical to that of authentic GLA standard. Analysis
    of this peak by gas chromatography-mass spectrometry
    (GC-MS) confirmed that it had the same mass
10 fragmentation pattern as a GLA reference sample.
    Transgenic Anabaena with altered levels of
    polyunsaturated fatty acids were similar to wild type
    in growth rate and morphology.

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1   Table 2 Composition of C18 Fatty Acids in Wild Type and Transgenic Cyanobacteria

	Fatty Acid (%)					
Strain	18:0	18:1	18:2	18.3(α)	18.3(γ)	18.4
Wild Type						
Synechocystis (sp.PCC6803)	13.6	4.5	54.5	–	27.3	–
Anabaena (sp.PCC7120)	2.9	24.8	37.1	35.2	–	–
Synechococcus (sp.PCC7942)	20.6	79.4	–	–	–	–
Anabaena Transconjugants						
cSy75	3.8	24.4	22.3	9.1	27.9	12.5
cSy75-3.5	4.3	27.6	18.1	3.2	40.4	6.4
pAM542 - 1.8F	4.2	13.9	12.1	19.1	25.4	25.4
pAM542 - 1.8R	7.7	23.1	38.4	30.8	–	–
pAM542 - 1.7F	2.8	27.8	36.1	33.3	–	–
pAM542 - 1.7R	2.8	25.4	42.3	29.6	–	–
Synechococcus Transformants						
pAM854	27.8	72.2	–	–	–	–
pAM854 -Δ^{12}	4.0	43.2	46.0	–	–	–
pAM854 -Δ^6	18.2	81.8	–	–	–	–
pAM854 -Δ^6&Δ^{12}	42.7	25.3	19.5	–	16.5	–

18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3(α), linolenic acid; 18:3(γ), γ-linolenic acid; 18:4, octadecatetraenoic acid

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1   EXAMPLE 4
                        Transformation of Synechococcus
                     with △6 and △12 Desaturase Genes

5   A third cosmid, cSy7, which contains a △12-
        desaturase gene, was isolated by screening the
        Synechocystis genomic library with a oligonucleotide
        synthesized from the published Synechocystis △12-
        desaturase gene sequence (Wada et al. [1990] Nature
10   (London) 347, 200-203). A 1.7 kb AvaI fragment from
        this cosmid containing the △12-desaturase gene was
        identified and used as a probe to demonstrate that
        cSy13 not only contains a △6-desaturase gene but also
        a △12-desaturase gene (Figure 3). Genomic Southern
        blot analysis further showed that both the △6-and △12-
15   desaturase genes are unique in the Synechocystis
        genome so that both functional genes involved in C18
        fatty acid desaturation are linked closely in the
        Synechocystis genome.

            The unicellular cyanobacterium Synechococcus
20   (PCC 7942) is deficient in both linoleic acid and
        GLA(3). The △12 and △6-desaturase genes were cloned
        individually and together into pAM854 (Bustos et al.
        [1991] J. Bacteriol. 174, 7525-7533), a shuttle vector
        that contains sequences necessary for the integration
25   of foreign DNA into the genome of Synechococcus
        (Golden et al. [1987] Methods in Enzymol. 153, 215-
        231). Synechococcus was transformed with these gene
        constructs and colonies were selected. Fatty acid
        methyl esters were extracted from transgenic
30   Synechococcus and analyzed by GLC.

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1   Table 2 shows that the principal fatty acids
    of wild type Synechococcus are stearic acid (18:0) and
    oleic acid (18:1). Synechococcus transformed with
    pAM854-Δ12 expressed linoleic acid (18:2) in addition
5   to the principal fatty acids. Transformants with
    pAM854-Δ6 and Δ12 produced both linoleate and GLA
    (Table 1). These results indicated that Synechococcus
    containing both Δ12- and Δ6-desaturase genes has
    gained the capability of introducing a second double
10 bond at the Δ12 position and a third double bond at
    the Δ6 position of C18 fatty acids. However, no
    changes in fatty acid composition was observed in the
    transformant containing pAM854-Δ6, indicating that in
    the absence of substrate synthesized by the Δ12
15 desaturase, the Δ6-desaturase is inactive. This
    experiment further confirms that the 1.8 kb
    NheI/HindIII fragment (Figure 3) contains both coding
    and promoter regions of the Synechocystis Δ6-
    desaturase gene. Transgenic Synechococcus with
20 altered levels of polyunsaturated fatty acids were
    similar to wild type in growth rate and morphology.

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1   **EXAMPLE 5**

                 Nucleotide Sequence of Δ6-Desaturase

              The nucleotide sequence of the 1.8 kb

5   fragment of cSy75-3.5 including the functional Δ6-

     desaturase gene was determined. An open reading frame

     encoding a polypeptide of 359 amino acids was

     identified (Figure 4). A Kyte-Doolittle hydropathy

     analysis (Kyte et al. [1982] J. Mol. Biol. 157, 105-

10   132) identified two regions of hydrophobic amino acids

     that could represent transmembrane domains (Figure

     1A); furthermore, the hydropathic profile of the Δ6-

     desaturase is similar to that of the Δ12-desaturase

     gene (Figure 1B; Wada et al.) and Δ9-desaturases

15   (Thiede et al. [1986] J. Biol. Chem. 261, 13230-

     13235). However, the sequence similarity between the

     Synechocystis Δ6- and Δ12-desaturases is less than 40%

     at the nucleotide level and approximately 18% at the

     amino acid level.

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1   **EXAMPLE 6**
           Transfer of Cyanobacterial Δ^6-Desaturase into Tobacco

                   The cyanobacterial Δ^6-desaturase gene was
5   mobilized into a plant expression vector and
      transferred to tobacco using <u>Agrobacterium</u> mediated
      gene transfer techniques. To ensure that the
      transferred desaturase is appropriately expressed in
      leaves and developing seeds and that the desaturase
10   gene product is targeted to the endoplasmic reticulum
      or the chloroplast, various expression cassettes with
      <u>Synechocystis</u> Δ-desaturase open reading frame (ORF)
      were constructed. Components of these cassettes
      include: (i) a 35S promoter or seed specific promoter
15   derived from the sunflower helianthinin gene to drive
      Δ^6-desaturase gene expression in all plant tissues or
      only in developing seeds respectively, (ii) a putative
      signal peptide either from carrot extensin gene or
      sunflower helianthinin gene to target newly
20   synthesized Δ^6-desaturase into the ER, (iii) an ER
      lumen retention signal sequence (KDEL) at the COOH-
      terminal of the Δ^6-desaturase ORF, and (iv) an
      optimized transit peptide to target Δ^6 desaturase into
      the chloroplast. The 35S promoter is a derivative of
25   pRTL2 described by Restrepo <u>et al</u>. (1990). The
      optimized transit peptide sequence is described by Van
      de Broeck <u>et al</u>. (1985). The carrot extensin signal
      peptide is described by Chen <u>et al</u> (1985) <u>EMBO J. 9</u>,
      2145.

30   Transgenic tobacco plants were produced
      containing a chimeric cyanobacterial desaturase gene,

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1   comprised of the _Synechocystis_ Δ⁶ desaturase gene fused
    to an endoplasmic reticulum retention sequence (KDEL)
    and extensin signal peptide driven by the CaMV 35S
    promoter. PCR amplifications of transgenic tobacco
5   genomic DNA indicate that the Δ⁶ desaturase gene was
    incorporated into the tobacco genome. Fatty acid
    methyl esters of leaves of these transgenic tobacco
    plants were extracted and analyzed by Gas Liquid
    Chromatography (GLC). These transgenic tobacco
10 accumulated significant amounts of GLA (Figure 4).
    Figure 4 shows fatty acid methyl esters as determined
    by GLC. Peaks were identified by comparing the
    elution times with known standards of fatty acid
    methyl ester. Accordingly, cyanobacterial genes
15 involved in fatty acid metabolism can be used to
    generate transgenic plants with altered fatty acid
    compositions.

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1   EXAMPLE 7
                 Construction of Borage cDNA library

         Membrane bound polysomes were isolated from
5 borage seeds 12 days post pollination (12 DPP) using
   the protocol established for peas by Larkins and
   Davies (1975 <u>Plant Phys</u>. 55:749-756). RNA was
   extracted from the polysomes as described by Mechler
   (1987 Methods in Enzymology <u>152</u>:241-248, Academic
10 Press).
         Poly-A+ RNA was isolated from the membrane
   bound polysomal RNA by use of Oligotex-dT beads
   (Qiagen). Corresponding cDNA was made using
   Stratagene's ZAP cDNA synthesis kit. The cDNA library
15 was constructed in the lambda ZAP II vector
   (Stratagene) using the lambda ZAP II vector kit. The
   primary library was packaged in Gigapack II Gold
   packaging extract (Stratagene). The library was used
   to generate expressed sequence tags (ESTs), and
20 sequences corresponding to the tags were used to scan
   the GenBank database.

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1   **EXAMPLE 8**
                              Hybridization Protocol

            Hybridization probes for screening the
5   borage cDNA library were generated by using random
      primed DNA synthesis as described by Ausubel et al
      (1994 Current Protocols in Molecular Biology, Wiley
      Interscience, N.Y.) and corresponded to previously
      identified abundantly expressed seed storage protein
10   cDNAs. Unincorporated nucleotides were removed by use
      of a G-50 spin column (Boehringer Manheim). Probe was
      denatured for hybridization by boiling in a water bath
      for 5 minutes, then quickly cooled on ice. Filters
      for hybridization were prehybridized at 60°C for 2-4
15   hours in prehybridization solution (6XSSC [Maniatis et
      al 1984 Molecular Cloning A Laboratory Manual, Cold
      Spring Harbor Laboratory], 1X Denharts Solution, 0.05%
      sodium pyrophosphate, 100 μg/ml denatured salmon sperm
      DNA). Denatured probe was added to the hybridization
20   solution (6X SSC, 1X Denharts solution, 0.05% sodium
      pyrophosphate, 100 μg/ml denatured salmon sperm DNA)
      and incubated at 60°C with agitation overnight.
      Filters were washed in 4x, 2x, and 1x SET washes for
      15 minutes each at 60°C. A 20X SET stock solution is
25   3M NaCl, 0.4 M Tris base, 20 mM $Na_2EDTA-2H_2O$. The 4X
      SET wash was 4X SET, 12.5 mM PO_4, pH 6.8 and 0.2% SDS.
      The 2X SET wash was 2X SET, 12.5 mM PO_4, pH 6.8 and
      0.2% SDS. The 1X SET wash was 1X SET, 12.5 mM PO_4, pH
      6.8 and 0.2% SDS. Filters were allowed to air dry and
30   were then exposed to X-ray film for 24 hours with
      intensifying screens at -80°C.

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1   EXAMPLE 9
                   Random sequencing of cDNAs from a borage seed
                     (12 DPP) membrane-bound polysomal library

5   The borage cDNA library was plated at low
        density (500 pfu on 150 mm petri dishes). Highly
        prevalent seed storage protein cDNAs were "subtracted"
        by screening with the previously identified
        corresponding cDNAs. Non-hybridizing plaques were
10   excised using Stratagene's excision protocol and
        reagents. Resulting bacterial colonies were used to
        inoculate liquid cultures and were either sequenced
        manually or by an ABI automated sequencer. Each cDNA
        was sequenced once and a sequence tag generated from
        200-300 base pairs. All sequencing was performed by
15   cycle sequencing (Epicentre). Over 300 ESTs were
        generated. Each sequence tag was compared to GenBank
        database by BLASTX computer program and a number of
        lipid metabolism genes, including the Δ6-desaturase
        were identified.
20   Database searches with a cDNA clone
        designated mbp-65 using BLASTX with the GenBank
        database resulted in a significant match to the
        Synechocystis Δ6-desaturase. It was determined
        however, that this clone was not a full length cDNA.
25   A full length cDNA was isolated using mbp-65 to screen
        the borage membrane-bound polysomal library. The
        sequence of the isolated cDNA was determined (Fig. 5A,
        SEQ ID NO:4) and the protein sequence of the open
        reading frame (Fig. 5B, SEQ ID NO:5) was compared to
30   other known desaturases using Geneworks

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1   (IntelligGenetics) protein alignment program (Fig. 2).
    This alignment indicated that the cDNA was the borage
    Δ6-desaturase gene.

           Although similar to other known plant
5   desaturases, the borage delta 6-desaturase is distinct
    as indicated in the dendrogram shown in Fig. 6.
    Furthermore, comparison of the amino acid sequences
    characteristic of desaturases, particularly those
    proposed to be involved in metal binding (metal box 1
10 and metal box 2), illustrates the differences between
    the borage delta 6-desaturase and other plant
    desaturases (Table 3).

           The borage delta 6-desaturase is
    distinguished from the cyanobacterial form not only in
15 over all sequence (Fig. 6) but also in the lipid box,
    metal box 1 and metal box 2 amino acid motifs (Table
    3). As Table 3 indicates, all three motifs are novel
    in sequence. Only the borage delta 6-desaturase metal
    box 2 shown some relationship to the Synechocystis
20 delta-6 desaturase metal box 2.

           In addition, the borage delta 6-desaturase
    is also distinct from another borage desaturase gene,
    the delta-12 desaturase. P1-81 is a full length cDNA
    that was identified by EST analysis and shows high
25 similarity to the Arabidopsis delta-12 desaturase (Fad
    2). A comparison of the lipid box, metal box 1 and
    metal box 2 amino acid motifs (Table 3) in borage
    delta 6 and delta-12 desaturases indicates that little
    homology exists in these regions. The placement of
30 the two sequences in the dendrogram in Fig. 6
    indicates how distantly related these two genes are.

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Table 3. Comparison of common amino acid motifs in membrane-bound desaturases

Desaturase	Lipid Box	Amino Acid Motif — Metal Box 1	Metal Box 2
Borage Δ⁶	WIGHDAGH (SEQ. ID. NO: 6)	HNAHH (SEQ. ID. NO: 12)	FQIEHH (SEQ. ID. NO: 20)
Synechocystis Δ⁶	NVGHDANH (SEQ. ID. NO: 7)	HNYLHH (SEQ. ID. NO: 13)	HQVTHH (SEQ. ID. NO: 21)
Arab. chloroplast Δ¹⁵	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVIHH (SEQ. ID. NO: 22)
Rice Δ¹⁵	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVIHH (SEQ. ID. NO: 22)
Glycine chloroplast Δ¹⁵	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVIHH (SEQ. ID. NO: 22)
Arab. fad3 (Δ¹⁵)	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVIHH (SEQ. ID. NO: 22)
Brassica fad3 (Δ¹⁵)	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVIHH (SEQ. ID. NO: 22)
Borage Δ¹² (P1-81)*	VIAHECGH (SEQ. ID. NO: 9)	HRRHH (SEQ. ID. NO: 15)	HVAHH (SEQ. ID. NO: 23)
Arab. fad2 (Δ¹²)	VIAHECGH (SEQ. ID. NO: 9)	HRRHH (SEQ. ID. NO: 15)	HVAHH (SEQ. ID. NO: 23)
Arab. chloroplast Δ¹²	VIGHDCAH (SEQ. ID. NO: 10)	HDRHH (SEQ. ID. NO: 16)	HIPHH (SEQ. ID. NO: 24)
Glycine plastid Δ¹²	VIGHDCAH (SEQ. ID. NO: 10)	HDRHH (SEQ. ID. NO: 16)	HIPHH (SEQ. ID. NO: 24)
Spinach plastidial n-6	VIGHDCAH (SEQ. ID. NO: 10)	HDQHH (SEQ. ID. NO: 17)	HIPHH (SEQ. ID. NO: 24)
Synechocystis Δ¹²	VVGHDCGH (SEQ. ID. NO: 11)	HDHHH (SEQ. ID. NO: 18)	HIPHH (SEQ. ID. NO: 24)
Anabaena Δ¹²	VLGHDCGH (SEQ. ID. NO: 8)	HNHHH (SEQ. ID. NO: 19)	HVPHH (SEQ. ID. NO: 25)

*P1-81 is a full length cDNA which was identified by EST analysis and shows high similarity to the Arbidopsis Δ12 desaturase (fad2)

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EXAMPLE 10
Construction of 222.1Δ⁶NOS for transient and expression

5   The vector pBI221 (Jefferson et al. 1987
EMBO J. 6:3901-3907) was prepared for ligation by
digestion with BamHI and EcoICR I (Promega) which
excises the GUS coding region leaving the 35S promoter
and NOS terminator intact. The borage Δ 6-desaturase
cDNA was excised from the Bluescript plasmid
10 (Stratagene) by digestion with BamHI and XhoI. The
XhoI end was made blunt by use of the Klenow fragment.
This fragment was then cloned into the BamHI/EcoICR I
sites of pBI221, yielding 221.Δ⁶NOS (Fig. 7). In
221.Δ⁶.NOS, the remaining portion (backbone) of the
15 restriction map depicted in Fig. 7 is pBI221.

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1   **EXAMPLE 11**
           Construction of 121.Δ⁶.NOS for stable transformation

              The vector pBI121 (Jefferson et al. 1987
5   EMBO J. 6:3901-3907) was prepared for ligation by
     digestion with BamHI and EcoICR I (Promega) which
     excises the GUS coding region leaving the 35S promoter
     and NOS terminator intact. The borage Δ 6-desaturase
     cDNA was excised from the Bluescript plasmid
10   (Stratagene) by digestion with BamHI and XhoI. The
     XhoI end was made blunt by use of the Klenow fragment.
     This fragment was then cloned into the BamHI/EcoICR I
     sites of pBI121, yielding 121.1Δ⁶NOS (Fig. 7).   In
     121.Δ⁶.NOS, the remaining portion (backbone) of the
15   restriction map depicted in Fig. 7 is pBI121.

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EXAMPLE 12
Transient Expression

All work involving protoplasts was performed in a sterile hood. One ml of packed carrot suspension cells were digested in 30 mls plasmolyzing solution (25 g/l KCl, 3.5 g/l $CaCl_2$-H_2O, 10mM MES, pH 5.6 and 0.2 M mannitol) with 1% cellulase, 0.1% pectolyase, and 0.1% dreisalase overnight, in the dark, at room temperature. Released protoplasts were filtered through a 150 μm mesh and pelleted by centrifugation (100x g, 5 min.) then washed twice in plasmolyzing solution. Protoplasts were counted using a double chambered hemocytometer. DNA was transfected into the protoplasts by PEG treatment as described by Nunberg and Thomas (1993 <u>Methods in Plant Molecular Biology and Biotechnology</u>, B.R. Glick and J.E. Thompson, eds. pp. 241-248) using 10^6 protoplasts and 50-70 ug of plasmid DNA (221.Δ6.NOS). Protoplasts were cultured in 5 mls of MS media supplemented with 0.2M mannitol and 3 μm 2,4-D for 48 hours in the dark with shaking.

1   **EXAMPLE 13**
                        Stable transformation of tobacco

        121.Δ^6.NOS plasmid construction was used to
5   transform tobacco (Nicotiana tabacum cv. xanthi) via
    Agrobacterium according to standard procedures (Horsh
    et al., 1985 Science _227_: 1229-1231; Bogue et al.,
    1990 Mol. Gen. Genet. _221_:49-57), except that initial
    transformants were selected on 100 ug/ml kanamycin.

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EXAMPLE 14
Preparation and analysis of
fatty acid methyl esters (FAMEs)

5   Tissue from transfected protoplasts and
transformed tobacco plants was frozen in liquid
nitrogen and lyophilized overnight. FAMEs were
prepared as described by Dahmer et al (1989 J. Amer.
Oil Chem. Soc. 66:543-548). In some cases, the
solvent was evaporated again, and the FAMEs were
10 resuspended in ethyl acetate and extracted once with
deionized water to remove any water soluble
contaminants. The FAMEs were analyzed by gas
chromatography (GC) on a J&W Scientific DB-wax column
(30 m length, 0.25 mm ID, 0.25 um film).
15   An example of a transient assay is shown in
Fig. 8 which represents three independent
transfections pooled together. The addition of the
borage Δ6-desaturase cDNA corresponds with the
appearance of gamma linolenic acid (GLA) which is one
20 of the possible products of Δ6-desaturase.
          Figures 9 and 10 depict GC profiles of the
FAMES derived from leaf and seed tissue, respectively,
of control and transformed tobacco plants. Figure 9A
provides the profile of leaf tissue of wild-type
25 tobacco (xanthi); Figure 9B provides the profile of
leaf tissue from a tobacco plant transformed with the
borage Δ-6 desaturase under the transcriptional
control of the 35S CaMV promoter (pBI 121Δ'NOS).
Peaks correspond to 18:2, 18:3γ (GLA), 18:3α and 18:4
30 (octadecanonic acid). Figure 10A shows the GC profile
of seeds of a wild-type tobacco; Figure 10B shows the

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1 profile of seed tissue of a tobacco plant transformed
   with pBI 121Δ⁶NOS. Peaks correspond to 18:2,
   18:3γ(GLA) and 18:3α.

          The relative distribution of the C_{18} fatty
5 acids in control and transgenic tobacco seeds is shown
   in Table 4.

<div align="center">TABLE 4</div>

Fatty Acid	Xanthi	pBI121Δ⁶NOS
18:0	4.0%	2.5%
18:1	13%	13%
18:2	82%	82%
18:3γ (GLA)		2.7%
18:3α	0.82%	1.4%

          The foregoing results demonstrate that GLA
   is incorporated into the triacylglycerides of
   transgenic tobacco leaves and seeds containing the
20 borage Δ6-desaturase.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT: Rhone-Poulenc Agrochimie

    (ii) TITLE OF INVENTION: PRODUCTION OF GAMMA LINOLENIC ACID BY A
                 DELTA 6-DESATURASE

    (iii) NUMBER OF SEQUENCES: 25

    (iv) CORRESPONDENCE ADDRESS:

        (A) ADDRESSEE: Scully, Scott, Murphy & Presser
        (B) STREET: 400 Garden City Plaza
        (C) CITY: Garden City
        (D) STATE: New York
        (E) COUNTRY: United States
        (F) ZIP: 11530

    (v) COMPUTER READABLE FORM:

        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

    (vi) CURRENT APPLICATION DATA:

        (A) APPLICATION NUMBER:
        (B) FILING DATE: 30-DEC-1994
        (C) CLASSIFICATION:

    (viii) ATTORNEY/AGENT INFORMATION:

        (A) NAME: Presser, Leopold
        (B) REGISTRATION NUMBER: 19,827
        (C) REFERENCE/DOCKET NUMBER: 8383ZYXW

    (ix) TELECOMMUNICATION INFORMATION:

        (A) TELEPHONE: (516) 742-4343
        (B) TELEFAX: (516) 742-4366
        (C) TELEX: 230 901 SANS UR

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 3588 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: both
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

    (A) NAME/KEY: CDS
    (B) LOCATION: 2002..3081

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GCTAGCCACC AGTGACGATG CCTTGAATTT GGCCATTCTG ACCCAGGCCC GTATTCTGAA      60

TCCCCGCATT CGCATTGTTA ATCGTTTGTT CAACCATGCC CTGGGTAAAC GTTTAGACAC     120

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TGCGGCTTTG GGCAATCAGG CGATCGGGCA ATTGCGTTTG TTTGACCAGA CTTGGCCCAT     240

TCAGGAAATT GTCATTCACC AAGACCATCC CTGGCTCAAT TTACCCCTGG CGGATTTATG     300

GGATGATCCG AGCCGAATGT TGATCTATTA CCTACCGGCC CACAGTGAAA CGGATTTAGT     360

AGGCGCAGTG GTGAATAATT TAACGTTGCA ATCTGGGGAC CATTTAATAG TGGGACAAAA     420

ACCCCAACCC AAGACCAAAC GGCGATCGCC TTGGCGCAAA TTTTCCAAAC TGATTACCAA     480

CCTGCGGGAG TATCAGCGGT ATGTCCAACA GGTGATATGG GTGGTGTTGT TTTTATTGTT     540

GATGATTTTT CTGGCCACCT TCATCTACGT TTCCATTGAT CAACATATTG CCCCAGTGGA     600

CGCGTTGTAT TTTTCCGTGG GCATGATTAC CGGGGCCGGT GGCAAGGAAG AGGTGGCCGA     660

AAAGTCCCCC GATATCATCA AAGTATTCAC AGTGGTGATG ATGATCGCCG GGGCGGGGGT     720

GATTGGTATT TGTTATGCCC TACTGAATGA TTTCATCCTT GGCAGTCGCT TTAGTCAGTT     780

TTTGGATGCG GCCAAGTTAC CCGATCGCCA TCACATCATC ATTTGTGGGC TGGGGGGAGT     840

GAGCATGGCC ATTATTGAAG AGTTAATTCA CCAGGGCCAT GAAATTGTGG TAATCGAAAA     900

GGATACAGAT AATCGTTTCT TGCATACGGC CCGCTCCCTG GGGGTGCCCG TAATTGTGGA     960

GGATGCCCGC CTAGAAAGAA CGTTGGCCTG CGCCAATATC AACCGAGCCG AAGCCATTGT    1020

GGTGGCCACC AGCGACGACA CCGTTAACTT GGAAATTGGC CTAACTGCCA AGGCGATCGC    1080

CCCTAGCCTG CCAGTGGTGT TGCGTTGCCA GGATGCCCAG TTTAGCCTGT CCCTGCAGGA    1140

AGTATTTGAA TTTGAAACGG TGCTTTGTCC GGCGGAATTG GCCACCTATT CCTTTGCGGC    1200

GGCGGCCCTG GGGGGCAAAA TTTTGGGCAA CGGCATGACC GATGATTTGC TGTGGGTAGC    1260

CCTAGCCACC TTAATCACTC CTAACCATCC CTTTGCCGAC CAATTGGTTA AAATTGCAGC    1320

CCAAAAGTCT GATTTCGTTC CCCTCTATCT AGAACGGGGT GGCAAAACCA TCCATAGCTG    1380

GGAATTATTG GGTACCCATC TCGACTCTGG AGACGTGTTG TATTTAACCA TGCCCGCCAC    1440

TGCCCTAGAG CAACTTTGGC GATCGCCCCG TGCCACTGCT GATCCTCTGG ACTCTTTTTT    1500
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GGTTTAGCAT GGGGGGATGG AACTCTTGAC TCGGCCCAAT GGTGATCAAG AAAGAACGCT    1560

TTGTCTATGT TTAGTATTTT TAAGTTAACC AACAGCAGAG GATAACTTCC AAAAGAAATT    1620

AAGCTCAAAA AGTAGCAAAA TAAGTTTAAT TCATAACTGA GTTTTACTGC TAAACAGCGG    1680

TGCAAAAAAG TCAGATAAAA TAAAAGCTTC ACTTCGGTTT TATATTGTGA CCATGGTTCC    1740

CAGGCATCTG CTCTAGGGAG TTTTTCCGCT GCCTTTAGAG AGTATTTTCT CCAAGTCGGC    1800

TAACTCCCCC ATTTTTAGGC AAAATCATAT ACAGACTATC CCAATATTGC CAGAGCTTTG    1860

ATGACTCACT GTAGAAGGCA GACTAAAATT CTAGCAATGG ACTCCCAGTT GGAATAAATT    1920

TTTAGTCTCC CCCGGCGCTG GAGTTTTTTT GTAGTTAATG GCGGTATAAT GTGAAAGTTT    1980
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TTTATCTATT TAAATTTATA A ATG CTA ACA GCG GAA AGA ATT AAA TTT ACC      2031
                         Met Leu Thr Ala Glu Arg Ile Lys Phe Thr
                          1                   5                  10

CAG AAA CGG GGG TTT CGT CGG GTA CTA AAC CAA CGG GTG GAT GCC TAC      2079
Gln Lys Arg Gly Phe Arg Arg Val Leu Asn Gln Arg Val Asp Ala Tyr
                 15                  20                  25

TTT GCC GAG CAT GGC CTG ACC CAA AGG GAT AAT CCC TCC ATG TAT CTG     2127
Phe Ala Glu His Gly Leu Thr Gln Arg Asp Asn Pro Ser Met Tyr Leu
             30                  35                  40

AAA ACC CTG ATT ATT GTG CTC TGG TTG TTT TCC GCT TGG GCC TTT GTG     2175
Lys Thr Leu Ile Ile Val Leu Trp Leu Phe Ser Ala Trp Ala Phe Val
             45                  50                  55

CTT TTT GCT CCA GTT ATT TTT CCG GTG CGC CTA CTG GGT TGT ATG GTT     2223
Leu Phe Ala Pro Val Ile Phe Pro Val Arg Leu Leu Gly Cys Met Val
         60                  65                  70

TTG GCG ATC GCC TTG GCG GCC TTT TCC TTC AAT GTC GGC CAC GAT GCC     2271
Leu Ala Ile Ala Leu Ala Ala Phe Ser Phe Asn Val Gly His Asp Ala
75                  80                  85                  90

AAC CAC AAT GCC TAT TCC TCC AAT CCC CAC ATC AAC CGG GTT CTG GGC     2319
Asn His Asn Ala Tyr Ser Ser Asn Pro His Ile Asn Arg Val Leu Gly
                 95                  100                 105

ATG ACC TAC GAT TTT GTC GGG TTA TCT AGT TTT CTT TGG CGC TAT CGC     2367
Met Thr Tyr Asp Phe Val Gly Leu Ser Ser Phe Leu Trp Arg Tyr Arg
             110                 115                 120

CAC AAC TAT TTG CAC CAC ACC TAC ACC AAT ATT CTT GGC CAT GAC GTG     2415
His Asn Tyr Leu His His Thr Tyr Thr Asn Ile Leu Gly His Asp Val
             125                 130                 135

GAA ATC CAT GGA GAT GGC GCA GTA CGT ATG AGT CCT GAA CAA GAA CAT     2463
Glu Ile His Gly Asp Gly Ala Val Arg Met Ser Pro Glu Gln Glu His
140                 145                 150
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GTT GGT ATT TAT CGT TTC CAG CAA TTT TAT ATT TGG GGT TTA TAT CTT        2511
Val Gly Ile Tyr Arg Phe Gln Gln Phe Tyr Ile Trp Gly Leu Tyr Leu
155             160                 165                 170

TTC ATT CCC TTT TAT TGG TTT CTC TAC GAT GTC TAC CTA GTG CTT AAT        2559
Phe Ile Pro Phe Tyr Trp Phe Leu Tyr Asp Val Tyr Leu Val Leu Asn
                175                 180                 185

AAA GGC AAA TAT CAC GAC CAT AAA ATT CCT CCT TTC CAG CCC CTA GAA        2607
Lys Gly Lys Tyr His Asp His Lys Ile Pro Pro Phe Gln Pro Leu Glu
                190                 195                 200

TTA GCT AGT TTG CTA GGG ATT AAG CTA TTA TGG CTC GGC TAC GTT TTC        2655
Leu Ala Ser Leu Leu Gly Ile Lys Leu Leu Trp Leu Gly Tyr Val Phe
                205                 210                 215

GGC TTA CCT CTG GCT CTG GGC TTT TCC ATT CCT GAA GTA TTA ATT GGT        2703
Gly Leu Pro Leu Ala Leu Gly Phe Ser Ile Pro Glu Val Leu Ile Gly
        220                 225                 230

GCT TCG GTA ACC TAT ATG ACC TAT GGC ATC GTG GTT TGC ACC ATC TTT        2751
Ala Ser Val Thr Tyr Met Thr Tyr Gly Ile Val Val Cys Thr Ile Phe
235             240                 245                 250

ATG CTG GCC CAT GTG TTG GAA TCA ACT GAA TTT CTC ACC CCC GAT GGT        2799
Met Leu Ala His Val Leu Glu Ser Thr Glu Phe Leu Thr Pro Asp Gly
                255                 260                 265

GAA TCC GGT GCC ATT GAT GAC GAG TGG GCT ATT TGC CAA ATT CGT ACC        2847
Glu Ser Gly Ala Ile Asp Asp Glu Trp Ala Ile Cys Gln Ile Arg Thr
                270                 275                 280

ACG GCC AAT TTT GCC ACC AAT AAT CCC TTT TGG AAC TGG TTT TGT GGC        2895
Thr Ala Asn Phe Ala Thr Asn Asn Pro Phe Trp Asn Trp Phe Cys Gly
                285                 290                 295

GGT TTA AAT CAC CAA GTT ACC CAC CAT CTT TTC CCC AAT ATT TGT CAT        2943
Gly Leu Asn His Gln Val Thr His His Leu Phe Pro Asn Ile Cys His
        300                 305                 310

ATT CAC TAT CCC CAA TTG GAA AAT ATT ATT AAG GAT GTT TGC CAA GAG        2991
Ile His Tyr Pro Gln Leu Glu Asn Ile Ile Lys Asp Val Cys Gln Glu
315             320                 325                 330

TTT GGT GTG GAA TAT AAA GTT TAT CCC ACC TTC AAA GCG GCG ATC GCC        3039
Phe Gly Val Glu Tyr Lys Val Tyr Pro Thr Phe Lys Ala Ala Ile Ala
                335                 340                 345

TCT AAC TAT CGC TGG CTA GAG GCC ATG GGC AAA GCA TCG TGACATTGCC         3088
Ser Asn Tyr Arg Trp Leu Glu Ala Met Gly Lys Ala Ser
                350                 355                 360

TTGGGATTGA AGCAAAATGG CAAAATCCCT CGTAAATCTA TGATCGAAGC CTTTCTGTTG      3148

CCCGCCGACC AAATCCCCGA TGCTGACCAA AGGTTGATGT TGGCATTGCT CCAAACCCAC      3208
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TTTGAGGGGG TTCATTGGCC GCAGTTTCAA GCTGACCTAG GAGGCAAAGA TTGGGTGATT    3268

TTGCTCAAAT CCGCTGGGAT ATTGAAAGGC TTCACCACCT TTGGTTTCTA CCCTGCTCAA    3328

TGGGAAGGAC AAACCGTCAG AATTGTTTAT TCTGGTGACA CCATCACCGA CCCATCCATG    3388

TGGTCTAACC CAGCCCTGGC CAAGGCTTGG ACCAAGGCCA TGCAAATTCT CCACGAGGCT    3448

AGGCCAGAAA AATTATATTG GCTCCTGATT TCTTCCGGCT ATCGCACCTA CCGATTTTTG    3508

AGCATTTTTG CCAAGGAATT CTATCCCCAC TATCTCCATC CCACTCCCCC GCCTGTACAA    3568

AATTTTATCC ATCAGCTAGC                                                3588
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(2) INFORMATION FOR SEQ ID NO:2:

      (i) SEQUENCE CHARACTERISTICS:

          (A) LENGTH: 359 amino acids
          (B) TYPE: amino acid
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: protein

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Leu Thr Ala Glu Arg Ile Lys Phe Thr Gln Lys Arg Gly Phe Arg
 1               5                  10                 15

Arg Val Leu Asn Gln Arg Val Asp Ala Tyr Phe Ala Glu His Gly Leu
            20              25                 30

Thr Gln Arg Asp Asn Pro Ser Met Tyr Leu Lys Thr Leu Ile Ile Val
        35              40                 45

Leu Trp Leu Phe Ser Ala Trp Ala Phe Val Leu Phe Ala Pro Val Ile
    50              55                 60

Phe Pro Val Arg Leu Leu Gly Cys Met Val Leu Ala Ile Ala Leu Ala
65          70                 75                 80

Ala Phe Ser Phe Asn Val Gly His Asp Ala Asn His Asn Ala Tyr Ser
            85              90                 95

Ser Asn Pro His Ile Asn Arg Val Leu Gly Met Thr Tyr Asp Phe Val
        100             105                110

Gly Leu Ser Ser Phe Leu Trp Arg Tyr Arg His Asn Tyr Leu His His
        115             120                125

Thr Tyr Thr Asn Ile Leu Gly His Asp Val Glu Ile His Gly Asp Gly
    130             135                140

Ala Val Arg Met Ser Pro Glu Gln Glu His Val Gly Ile Tyr Arg Phe
145             150                155                160
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Gln Gln Phe Tyr Ile Trp Gly Leu Tyr Leu Phe Ile Pro Phe Tyr Trp
            165             170             175

Phe Leu Tyr Asp Val Tyr Leu Val Leu Asn Lys Gly Lys Tyr His Asp
            180             185             190

His Lys Ile Pro Pro Phe Gln Pro Leu Glu Leu Ala Ser Leu Leu Gly
            195             200             205

Ile Lys Leu Leu Trp Leu Gly Tyr Val Phe Gly Leu Pro Leu Ala Leu
    210             215             220

Gly Phe Ser Ile Pro Glu Val Leu Ile Gly Ala Ser Val Thr Tyr Met
225             230             235             240

Thr Tyr Gly Ile Val Val Cys Thr Ile Phe Met Leu Ala His Val Leu
            245             250             255

Glu Ser Thr Glu Phe Leu Thr Pro Asp Gly Glu Ser Gly Ala Ile Asp
            260             265             270

Asp Glu Trp Ala Ile Cys Gln Ile Arg Thr Thr Ala Asn Phe Ala Thr
            275             280             285

Asn Asn Pro Phe Trp Asn Trp Phe Cys Gly Gly Leu Asn His Gln Val
    290             295             300

Thr His His Leu Phe Pro Asn Ile Cys His Ile His Tyr Pro Gln Leu
305             310             315             320

Glu Asn Ile Ile Lys Asp Val Cys Gln Glu Phe Gly Val Glu Tyr Lys
            325             330             335

Val Tyr Pro Thr Phe Lys Ala Ala Ile Ala Ser Asn Tyr Arg Trp Leu
            340             345             350

Glu Ala Met Gly Lys Ala Ser
            355
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(2) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 1884 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: both
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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AGCTTCACTT CGGTTTTATA TTGTGACCAT GGTTCCCAGG CATCTGCTCT AGGGAGTTTT        60

TCCGCTGCCT TTAGAGAGTA TTTTCTCCAA GTCGGCTAAC TCCCCCATTT TTAGGCAAAA       120
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TCATATACAG ACTATCCCAA TATTGCCAGA GCTTTGATGA CTCACTGTAG AAGGCAGACT    180

AAAATTCTAG CAATGGACTC CCAGTTGGAA TAAATTTTTA GTCTCCCCCG GCGCTGGAGT    240

TTTTTTGTAG TTAATGGCGG TATAATGTGA AAGTTTTTTA TCTATTTAAA TTTATAAATG    300

CTAACAGCGG AAAGAATTAA ATTTACCCAG AAACGGGGGT TTCGTCGGGT ACTAAACCAA    360

CGGGTGGATG CCTACTTTGC CGAGCATGGC CTGACCCAAA GGGATAATCC CTCCATGTAT    420

CTGAAAACCC TGATTATTGT GCTCTGGTTG TTTTCCGCTT GGGCCTTTGT GCTTTTTGCT    480

CCAGTTATTT TTCCGGTGCG CCTACTGGGT TGTATGGTTT TGGCGATCGC CTTGGCGGCC    540

TTTTCCTTCA ATGTCGGCCA CGATGCCAAC CACAATGCCT ATTCCTCCAA TCCCCACATC    600

AACCGGGTTC TGGGCATGAC CTACGATTTT GTCGGGTTAT CTAGTTTTCT TTGGCGCTAT    660

CGCCACAACT ATTTGCACCA CACCTACACC AATATTCTTG GCCATGACGT GGAAATCCAT    720

GGAGATGGCG CAGTACGTAT GAGTCCTGAA CAAGAACATG TTGGTATTTA TCGTTTCCAG    780

CAATTTTATA TTTGGGGTTT ATATCTTTTC ATTCCCTTTT ATTGGTTTCT CTACGATGTC    840

TACCTAGTGC TTAATAAAGG CAAATATCAC GACCATAAAA TTCCTCCTTT CCAGCCCCTA    900

GAATTAGCTA GTTTGCTAGG GATTAAGCTA TTATGGCTCG CTACGTTTT CGGCTTACCT     960

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CTCACCCCCG ATGGTGAATC CGGTGCCATT GATGACGAGT GGGCTATTTG CCAAATTCGT   1140

ACCACGGCCA ATTTTGCCAC CAATAATCCC TTTTGGAACT GGTTTTGTGG CGGTTTAAAT   1200

CACCAAGTTA CCCACCATCT TTTCCCCAAT ATTTGTCATA TTCACTATCC CCAATTGGAA   1260

AATATTATTA AGGATGTTTG CCAAGAGTTT GGTGTGGAAT ATAAAGTTTA TCCCACCTTC   1320

AAAGCGGCGA TCGCCTCTAA CTATCGCTGG CTAGAGGCCA TGGGCAAAGC ATCGTGACAT   1380

TGCCTTGGGA TTGAAGCAAA ATGGCAAAAT CCCTCGTAAA TCTATGATCG AAGCCTTTCT   1440

GTTGCCCGCC GACCAAATCC CCGATGCTGA CCAAAGGTTG ATGTTGGCAT TGCTCCAAAC   1500

CCACTTTGAG GGGGTTCATT GGCCGCAGTT TCAAGCTGAC CTAGGAGGCA AAGATTGGGT   1560

GATTTTGCTC AAATCCGCTG GGATATTGAA AGGCTTCACC ACCTTTGGTT TCTACCCTGC   1620

TCAATGGGAA GGACAAACCG TCAGAATTGT TTATTCTGGT GACACCATCA CCGACCCATC   1680

CATGTGGTCT AACCCAGCCC TGGCCAAGGC TTGGACCAAG GCCATGCAAA TTCTCCACGA   1740

GGCTAGGCCA GAAAAATTAT ATTGGCTCCT GATTTCTTCC GGCTATCGCA CCTACCGATT   1800
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TTTGAGCATT TTTGCCAAGG AATTCTATCC CCACTATCTC CATCCCACTC CCCCGCCTGT     1860

ACAAAATTTT ATCCATCAGC TAGC                                           1884
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(2) INFORMATION FOR SEQ ID NO:4:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 1685 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: both
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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AATATCTGCC TACCCTCCCA AAGAGAGTAG TCATTTTTCA TCAATGGCTG CTCAAATCAA       60

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GATTCAAGGG AAAGCCTATG ATGTTTCGGA TTGGGTGAAA GACCATCCAG GTGGCAGCTT      180

TCCCTTGAAG AGTCTTGCTG GTCAAGAGGT AACTGATGCA TTTGTTGCAT TCCATCCTGC      240

CTCTACATGG AAGAATCTTG ATAAGTTTTT CACTGGGTAT TATCTTAAAG ATTACTCTGT      300

TTCTGAGGTT TCTAAAGATT ATAGGAAGCT TGTGTTTGAG TTTTCTAAAA TGGGTTTGTA      360

TGACAAAAAA GGTCATATTA TGTTTGCAAC TTTGTGCTTT ATAGCAATGC TGTTTGCTAT      420

GAGTGTTTAT GGGGTTTTGT TTTGTGAGGG TGTTTTGGTA CATTTGTTTT CTGGGTGTTT      480

GATGGGGTTT CTTTGGATTC AGAGTGGTTG GATTGGACAT GATGCTGGGC ATTATATGGT      540

AGTGTCTGAT TCAAGGCTTA ATAAGTTTAT GGGTATTTTT GCTGCAAATT GTCTTTCAGG      600

AATAAGTATT GGTTGGTGGA AATGGAACCA TAATGCACAT CACATTGCCT GTAATAGCCT      660

TGAATATGAC CCTGATTTAC AATATATACC ATTCCTTGTT GTGTCTTCCA AGTTTTTTGG      720

TTCACTCACC TCTCATTTCT ATGAGAAAAG GTTGACTTTT GACTCTTTAT CAAGATTCTT      780

TGTAAGTTAT CAACATTGGA CATTTTACCC TATTATGTGT GCTGCTAGGC TCAATATGTA      840

TGTACAATCT CTCATAATGT TGTTGACCAA GAGAAATGTG TCCTATCGAG CTCAGGAACT      900

CTTGGGATGC CTAGTGTTCT CGATTTGGTA CCCGTTGCTT GTTTCTTGTT TGCCTAATTG      960

GGGTGAAAGA ATTATGTTTG TTATTGCAAG TTTATCAGTG ACTGGAATGC AACAAGTTCA     1020

GTTCTCCTTG AACCACTTCT CTTCAAGTGT TTATGTTGGA AAGCCTAAAG GGAATAATTG     1080

GTTTGAGAAA CAAACGGATG GGACACTTGA CATTTCTTGT CCTCCTTGGA TGGATTGGTT     1140

TCATGGTGGA TTGCAATTCC AAATTGAGCA TCATTTGTTT CCCAAGATGC CTAGATGCAA     1200
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CCTTAGGAAA ATCTCGCCCT ACGTGATCGA GTTATGCAAG AAACATAATT TGCCTTACAA   1260

TTATGCATCT TTCTCCAAGG CCAATGAAAT GACACTCAGA ACATTGAGGA ACACAGCATT   1320

GCAGGCTAGG GATATAACCA AGCCGCTCCC GAAGAATTTG GTATGGGAAG CTCTTCACAC   1380

TCATGGTTAA AATTACCCTT AGTTCATGTA ATAATTTGAG ATTATGTATC TCCTATGTTT   1440

GTGTCTTGTC TTGGTTCTAC TTGTTGGAGT CATTGCAACT TGTCTTTTAT GGTTTATTAG   1500

ATGTTTTTTA ATATATTTTA GAGGTTTTGC TTTCATCTCC ATTATTGATG AATAAGGAGT   1560

TGCATATTGT CAATTGTTGT GCTCAATATC TGATATTTTG GAATGTACTT TGTACCACTG   1620

TGTTTTCAGT TGAAGCTCAT GTGTACTTCT ATAGACTTTG TTTAAATGGT TATGTCATGT   1680

TATTT   1685

(2) INFORMATION FOR SEQ ID NO:5:

        (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 448 amino acids
             (B) TYPE: amino acid
             (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: DNA (genomic)

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

    Met Ala Ala Gln Ile Lys Lys Tyr Ile Thr Ser Asp Glu Leu Lys Asn
    1   5   10   15

        His Asp Lys Pro Gly Asp Leu Trp Ile Ser Ile Gln Gly Lys Ala Tyr
                    20   25   30

        Asp Val Ser Asp Trp Val Lys Asp His Pro Gly Gly Ser Phe Pro Leu
                    35   40   45

        Lys Ser Leu Ala Gly Gln Glu Val Thr Asp Ala Phe Val Ala Phe His
            50   55   60

        Pro Ala Ser Thr Trp Lys Asn Leu Asp Lys Phe Phe Thr Gly Tyr Tyr
        65   70   75   80

        Leu Lys Asp Tyr Ser Val Ser Glu Val Ser Lys Asp Tyr Arg Lys Leu
                        85   90   95

        Val Phe Glu Phe Ser Lys Met Gly Leu Tyr Asp Lys Lys Gly His Ile
                    100   105   110

        Met Phe Ala Thr Leu Cys Phe Ile Ala Met Leu Phe Ala Met Ser Val
                    115   120   125

        Tyr Gly Val Leu Phe Cys Glu Gly Val Leu Val His Leu Phe Ser Gly
            130   135   140

```
Cys Leu Met Gly Phe Leu Trp Ile Gln Ser Gly Trp Ile Gly His Asp
145             150             155             160

Ala Gly His Tyr Met Val Val Ser Asp Ser Arg Leu Asn Lys Phe Met
            165             170             175

Gly Ile Phe Ala Ala Asn Cys Leu Ser Gly Ile Ser Ile Gly Trp Trp
            180             185             190

Lys Trp Asn His Asn Ala His His Ile Ala Cys Asn Ser Leu Glu Tyr
        195             200             205

Asp Pro Asp Leu Gln Tyr Ile Pro Phe Leu Val Val Ser Ser Lys Phe
    210             215             220

Phe Gly Ser Leu Thr Ser His Phe Tyr Glu Lys Arg Leu Thr Phe Asp
225             230             235             240

Ser Leu Ser Arg Phe Phe Val Ser Tyr Gln His Trp Thr Phe Tyr Pro
            245             250             255

Ile Met Cys Ala Ala Arg Leu Asn Met Tyr Val Gln Ser Leu Ile Met
            260             265             270

Leu Leu Thr Lys Arg Asn Val Ser Tyr Arg Ala Gln Glu Leu Leu Gly
        275             280             285

Cys Leu Val Phe Ser Ile Trp Tyr Pro Leu Leu Val Ser Cys Leu Pro
    290             295             300

Asn Trp Gly Glu Arg Ile Met Phe Val Ile Ala Ser Leu Ser Val Thr
305             310             315             320

Gly Met Gln Gln Val Gln Phe Ser Leu Asn His Phe Ser Ser Ser Val
            325             330             335

Tyr Val Gly Lys Pro Lys Gly Asn Asn Trp Phe Glu Lys Gln Thr Asp
        340             345             350

Gly Thr Leu Asp Ile Ser Cys Pro Pro Trp Met Asp Trp Phe His Gly
        355             360             365

Gly Ser Gln Phe Gln Ile Glu His His Leu Phe Pro Lys Met Pro Arg
    370             375             380

Cys Asn Leu Arg Lys Ile Ser Pro Tyr Val Ile Glu Leu Cys Lys Lys
385             390             395             400

His Asn Leu Pro Tyr Asn Tyr Ala Ser Phe Ser Lys Ala Asn Glu Met
            405             410             415

Thr Leu Arg Thr Leu Arg Asn Thr Ala Leu Gln Ala Arg Asp Ile Thr
        420             425             430

Lys Pro Leu Pro Lys Asn Leu Val Trp Glu Ala Leu His Thr His Gly
        435             440             445
```

(2) INFORMATION FOR SEQ ID NO:6:

   (i) SEQUENCE CHARACTERISTICS:

      (A) LENGTH: 8 amino acids
      (B) TYPE: amino acid
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Trp Ile Gly His Asp Ala Gly His
1   5

(2) INFORMATION FOR SEQ ID NO:7:

   (i) SEQUENCE CHARACTERISTICS:

      (A) LENGTH: 8 amino acids
      (B) TYPE: amino acid
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Asn Val Gly His Asp Ala Asn His
1   5

(2) INFORMATION FOR SEQ ID NO:8:

   (i) SEQUENCE CHARACTERISTICS:

      (A) LENGTH: 8 amino acids
      (B) TYPE: amino acid
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Val Leu Gly His Asp Cys Gly His
1   5

(2) INFORMATION FOR SEQ ID NO:9:

   (i) SEQUENCE CHARACTERISTICS:

      (A) LENGTH: 8 amino acids
      (B) TYPE: amino acid
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

-55-

```
        Val Ile Ala His Glu Cys Gly His
        1               5
```

(2) INFORMATION FOR SEQ ID NO:10:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
        Val Ile Gly His Asp Cys Ala His
        1               5
```

(2) INFORMATION FOR SEQ ID NO:11:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
        Val Val Gly His Asp Cys Gly His
        1               5
```

(2) INFORMATION FOR SEQ ID NO:12:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 5 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
        His Asn Ala His His
        1               5
```

(2) INFORMATION FOR SEQ ID NO:13:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 6 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

-56-

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

His Asn Tyr Leu His His
1   5

(2) INFORMATION FOR SEQ ID NO:14:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 5 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

His Arg Thr His His
1   5

(2) INFORMATION FOR SEQ ID NO:15:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 5 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

His Arg Arg His His
1   5

(2) INFORMATION FOR SEQ ID NO:16:

        (i) SEQUENCE CHARACTERISTICS:

                (A) LENGTH: 5 amino acids
                (B) TYPE: amino acid
                (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA (genomic)

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

      His Asp Arg His His
      1   5

(2) INFORMATION FOR SEQ ID NO:17:

        (i) SEQUENCE CHARACTERISTICS:

                (A) LENGTH: 5 amino acids
                (B) TYPE: amino acid
                (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA (genomic)

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

      His Asp Gln His His
      1   5

(2) INFORMATION FOR SEQ ID NO:18:

        (i) SEQUENCE CHARACTERISTICS:

                (A) LENGTH: 5 amino acids
                (B) TYPE: amino acid
                (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA (genomic)

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

      His Asp His His His
      1   5

(2) INFORMATION FOR SEQ ID NO:19:

        (i) SEQUENCE CHARACTERISTICS:

                (A) LENGTH: 5 amino acids
                (B) TYPE: amino acid
                (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA (genomic)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

  His Asn His His His
  1   5

(2) INFORMATION FOR SEQ ID NO:20:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 6 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

  Phe Gln Ile Glu His His
  1   5

(2) INFORMATION FOR SEQ ID NO:21:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 6 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

  His Gln Val Thr His His
  1   5

(2) INFORMATION FOR SEQ ID NO:22:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 5 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

  His Val Ile His His
  1   5

(2) INFORMATION FOR SEQ ID NO:23:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 5 amino acids
        (B) TYPE: amino acid

                    (D) TOPOLOGY: linear

            (ii) MOLECULE TYPE: DNA (genomic)

            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

            His Val Ala His His
            1   5

    (2) INFORMATION FOR SEQ ID NO:24:

            (i) SEQUENCE CHARACTERISTICS:

                    (A) LENGTH: 5 amino acids
                    (B) TYPE: amino acid
                    (D) TOPOLOGY: linear

            (ii) MOLECULE TYPE: DNA (genomic)

            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

            His Ile Pro His His
            1   5

    (2) INFORMATION FOR SEQ ID NO:25:

            (i) SEQUENCE CHARACTERISTICS:

                    (A) LENGTH: 5 amino acids
                    (B) TYPE: amino acid
                    (D) TOPOLOGY: linear

            (ii) MOLECULE TYPE: DNA (genomic)

            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

            His Val Pro His His
            1   5

1   **WHAT IS CLAIMED**:

     1.   An isolated nucleic acid encoding a borage
Δ6-desaturase.

5

     2.   The isolated nucleic acid of Claim 1
comprising the nucleotide sequence of SEQ ID NO: 4.

     3.   An isolated nucleic acid that codes for the
10 amino acid sequence of SEQ ID NO: 5.

     4.   A vector comprising the nucleic acid of any
one Claims 1-3.

15   5.   An expression vector comprising the
isolated nucleic acid of any one of Claims 1-3 operably
linked to a promoter and optionally a termination signal
capable of effecting expression of the gene product of
said isolated nucleic acid.

20

     6.   The expression vector of Claim 5 wherein
said promoter is a Δ-6 desaturase promoter, an _Anabaena_
carboxylase promoter, a helianthinin promoter, a glycinin
promoter, a napin promoter, the 35S promoter from CaMV, or
a helianthinin tissue-specific promoter.
25

     7.   The expression vector of Claim 5 wherein
said promoter is constitutive or tissue-specific.

30   8.   The expression vector of Claim 5 wherein
said termination signal is a _Synechocystis_ termination

35

1   signal, a nopaline synthase termination signal, or a seed
     termination signal.

          9.   A cell comprising the vector of any one of
5   Claims 4-8.

          10.   The cell of Claim 9 wherein said cell is an
     animal cell, a bacterial cell, a plant cell or a fungal
     cell.
10

          11.   A transgenic organism comprising the
     isolated nucleic acid of any one of Claims 1-3.

          12.   A transgenic organism comprising the vector
15   of any one of Claims 4-8.

          13.   The transgenic organism of Claim 11 or 12
     wherein said organism is a bacterium, a fungus, a plant or
     an animal.
20

          14.   A plant or progeny of said plant which has
     been regenerated from the plant cell of Claim 10.

          15.   The plant of Claim 14 wherein said plant is
25   a sunflower, soybean, maize, tobacco, peanut, carrot or
     oil seed rape plant.

          16.   A method of producing a plant with
     increased gamma linolenic acid (GLA) content which
30   comprises:

35

1   (a) transforming a plant cell with the isolated
nucleic acid of any one of Claims 1-3; and
           (b) regenerating a plant with increased GLA
content from said plant cell.

5
           17.   A method of producing a plant with
increased gamma linolenic acid (GLA) content which
comprises:
           (a) transforming a plant cell with the vector of
10   any one of Claims 4-8; and
           (b) regenerating a plant with increased GLA
content from said plant cell.

           18.   The method of Claim 16 or 17 wherein said
15   plant is a sunflower, soybean, maize, tobacco, peanut,
carrot or oil seed rape plant.

           19.   A method of inducing production of gamma
linolenic acid (GLA) in an organism deficient or lacking
20   in GLA which comprises transforming said organism with the
isolated nucleic acid of any one of Claims 1-3.

           20.   A method of inducing production of gamma
linolenic acid (GLA) in an organism deficient or lacking
25   in GLA which comprises transforming said organism with the
vector of any one of Claims 4-8.

           21.   A method of inducing production of gamma
linolenic acid (GLA) in an organism deficient or lacking
30   in GLA and linoleic acid (LA) which comprises transforming
said organism with an isolated nucleic acid encoding

35

1   borage Δ6-desaturase and an isolated nucleic acid encoding
    Δ12-desaturase.

        22.   The method of Claim 21 wherein said
5   isolated nucleic acid encoding Δ6-desaturase comprises
    nucleotides 44 to 1390 of SEQ. ID NO: 4.

        23.   A method of inducing production of
    octadecatetraeonic acid in an organism deficient or
10 lacking in gamma linolenic acid which comprises
    transforming said organism with the isolated nucleic acid
    of any one of Claims 1-3.

        24.   A method of inducing production of
15 octadecatetraeonic acid in an organism deficient or
    lacking in gamma linolenic acid which comprises
    transforming said organism with the vector of any one of
    Claims 4-8.

        25.   The method of Claim 23 or 24 wherein said
20 organism is a bacterium, a fungus, a plant or an animal.

        26.   A method of producing a plant with improved
    chilling resistance which comprises:
        (a) transforming a plant cell with the isolated
25 nucleic acid of any one of Claims 1-3; and
        (b) regenerating said plant with improved
    chilling resistance from said transformed plant cell.

        27.   A method of producing a plant with improved
30 chilling resistance which comprises:

35

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1   (a) transforming a plant cell with the vector of
any one of Claims 4-8; and
          (b) regenerating said plant with improved
chilling resistance from said transformed plant cell.

5
          28. The method of Claim 26 or 27 wherein said
plant is a sunflower, soybean, maize, tobacco, peanut,
carrot or oil seed rape plant.

10

15

20

25

30

35

FIG. IA

FIG. IB

FIG. 2A

FIG. 2B

FIG. 3

Fatty Acid Methyl Esters from
Leaves of Tobacco (Wild Type)

FIG. 4A

Fatty Acid Methyl Esters from
Transgenic Tobacco(+Δ⁶)Desaturase
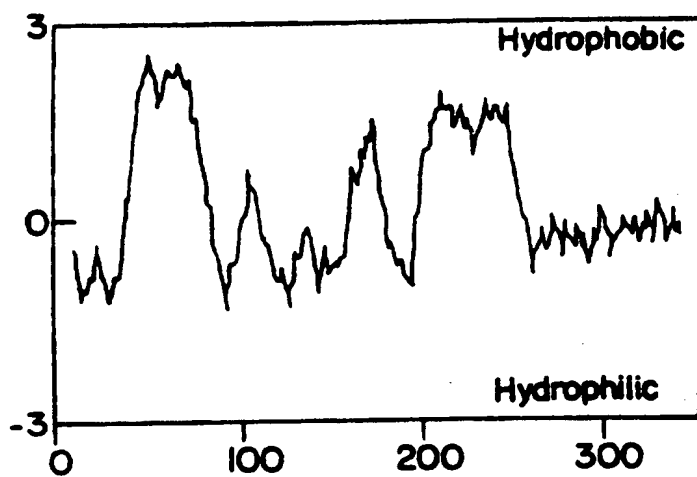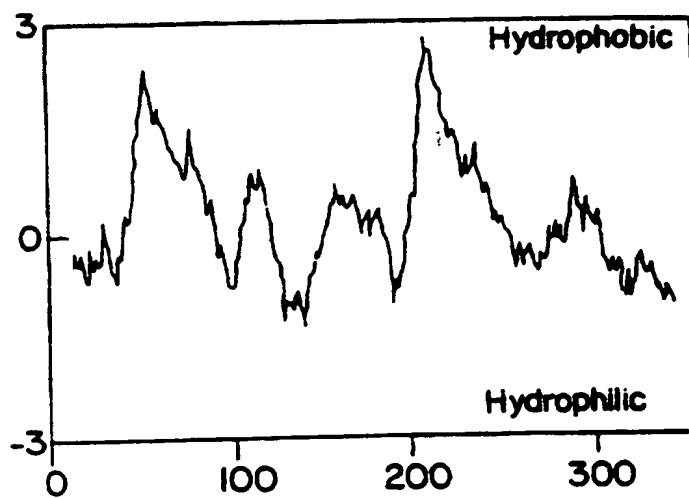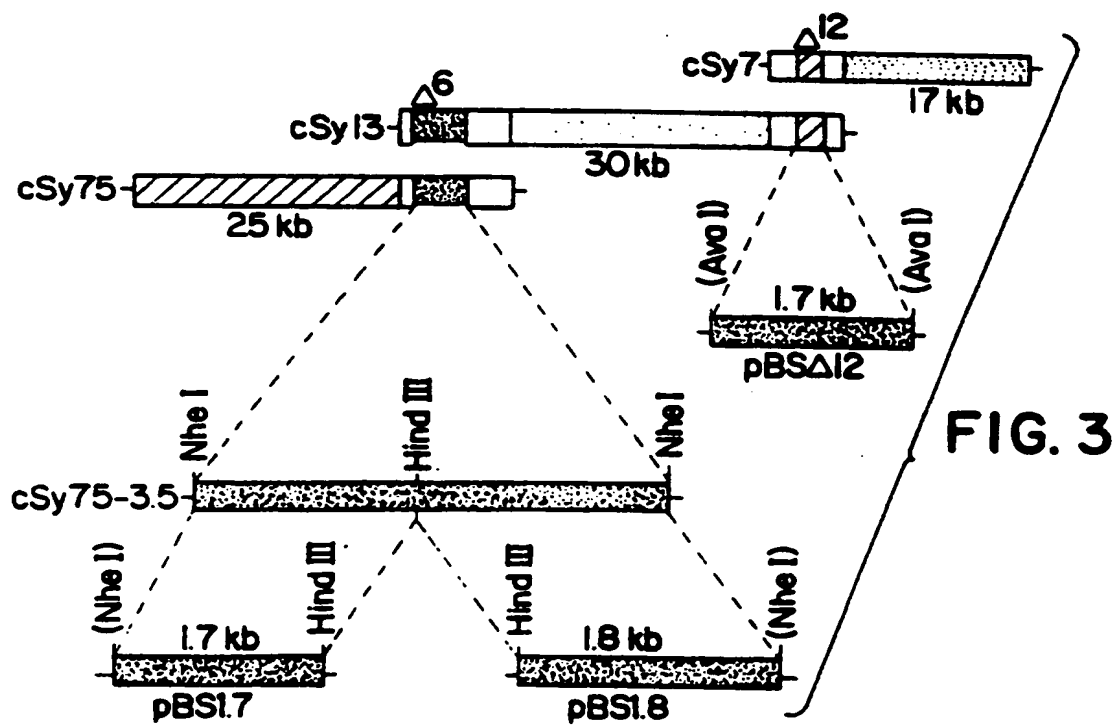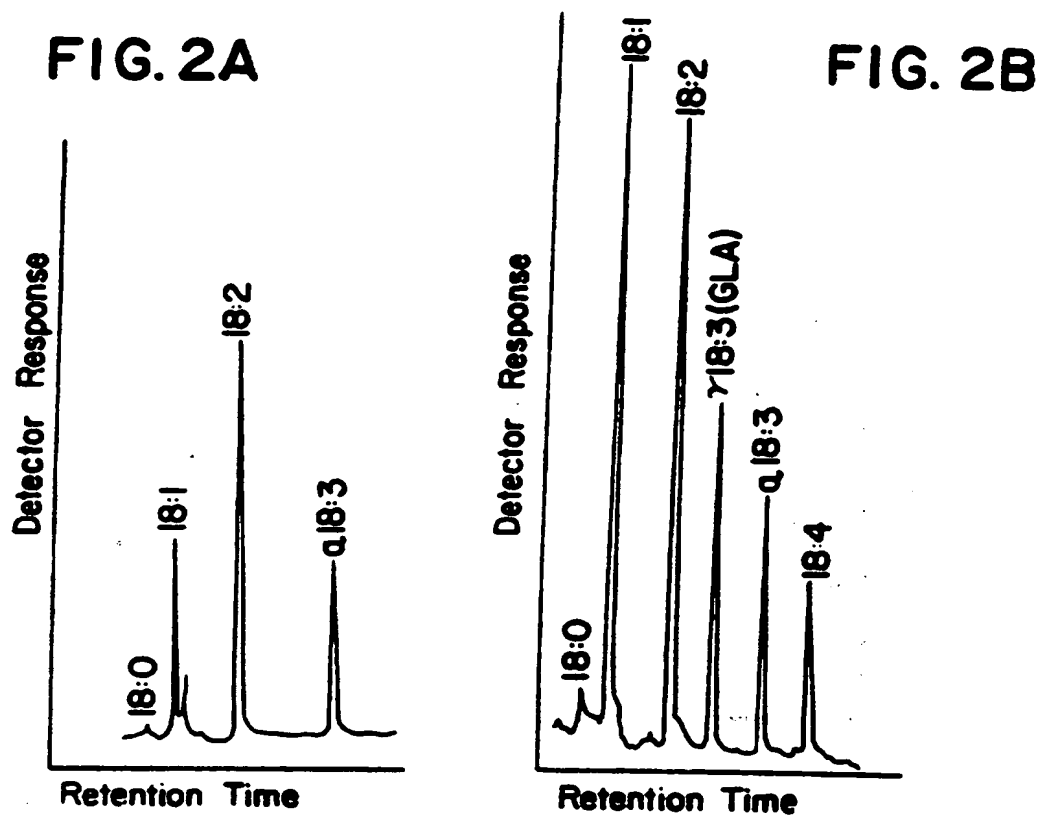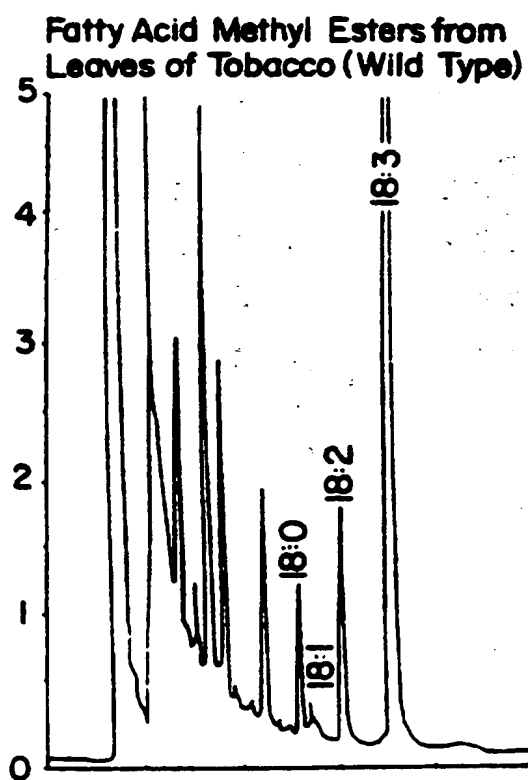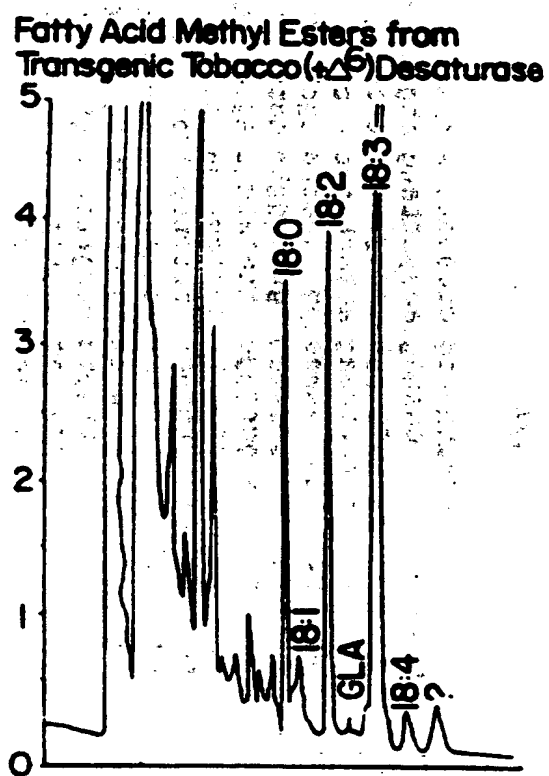
FIG. 4B

FIG.5A

```
   1  aatatctgcc taccctccca agagagtag  tcattttca  tcaatggctg ctcaaatcaa gaaatacatt acctcagatg    80
  81  aactcaagaa ccacgataaa cccggagatc tatgatctc  gattcaaggg aaagcctatg atgtttcgga ttgggtgaaa   160
 161  gaccatccag gtggcagctt tcccttgaag agtcttgctg gtcaagaggt aactgatgca tttgttgcat tccatcctgc   240
 241  ctctacatgg aagaatcttg ataagtttt  cactggtat  tatcttaaag attactctgt tctaaagatt tctgtgcttt   320
 321  ataggaagct tgtttgctat gagtgttgta tgggtttgt  tttgtgaggg tgtttggta  cattgtttt  ctggtgcttt   400
 401  atagcaatgc tgttggattc agagtggttg gattggacat gatgctggc  attatatggt agtgtctgat ctgggtgttt   480
 481  gatgggggttt cttggattc  agagtggttg gattggacat gatgctggc  agtgctggat agtgtctgat tcaaggctta   560
 561  ataagtttat gggtatttt  gctgcaaatt gtctttcagg aataagtatt ggttggtgga aatggaacca taatgcacat   640
 641  cacattgcct gtaatagcct cgaatatgac cctgatttac aatatatacc attccctgtt gtgtcttcca agttttttgg   720
 721  ttcactcacc tctcatttct tattatgtg  atgagaaaag gttgacttt  gactctttat caagatctt  caacattgga   800
 801  catttttcc  tattatgta  gctgctaggc tcaatatga  tgtacaatct ccgttgctt  gttcctgtt  tgcctaattg   880
 961  ggttgaaaga attatgtttg ttattgcaag tttatcagtg actggaatgc aacaagttca gttttcattg aaccacttct   960
1041  cttcaagtgt ttatgttgga aagcctaaag ggaataattg gtttgagaaa caaacggatg ggacacttga catttcctgt   1040
1121  cctcctgga  tcatggggtt tcatggtgga ttgcaattcc aaattgagca tcatttgttt cccagatgc  ctagatgcaa   1120
1201  cctaaggaaa atctcgccct acgtgatcga gttatgcaag aaacataatt tgccttacaa ttatgcatct tcctccaagg   1200
1281  ccaatgaaat gacactccac tcatggttaa gaaatactg  gcaggctagg ataataacca agccgctccc gaagaatttg   1280
1361  gtatggggaag ctcttcacac tcatggttaa aattccatga  agttccatga atatcttgag attatgtatc tcctatgttt   1360
1441  gtgtcctgtc ttggtccttc ttcatctctc attattgtg  tgcatattgt ggtttattag atgtttttta atatattta   1440
1521  gaggttttgc tttcatccctc cattaaggagt caattgttgt caattgttgt gctccaatacc tgatctttta   1520
1601  gaatgtactt tgtaccactg tgttttttcagt tgaagctcat gtgtacttct atagacttg gtgacttct  tatgtcatgt   1680
1681  tattt                                                                            1685
```

FIG.5B

```
  1  MAAQIKKYIT SDELKNHDKP GDLWISIQGK AYDVSDWVKD HPGGSFPLKS LAGQEVTDAF VAFHPASTWK NLDKFFTGYY    80

 81  LKDYSVSEVS KDYRKLVFEF SKMGLYDKKG HIMFATLCFI AMLFAMSVYG VLFCEGVLVH LFSGCLMGFL WIQSGWIGHD   160

161  AGHYMVVSDS RLNKFMGIFA ANCLSGISIG WWKWNHNAHH IACNSLEYDP DLQYIPFLVV SSKFFGSLTS HFYEKRLTFD   240

241  SLSRFFVSYQ HWTFYPIMCA ARLNMYVQSL IMLLTKRNVS YRAQELLGCL VFSIWYPLLV SCLPNWGERI MFVIASLSVT   320

321  GMQQVQFSLN HFSSSVYVGK PKGNNWFEKQ TDGTLDISCP PMDWFHGGL  QOIEHHLFP  KMPRCNLRKI SPYVIELCKK   400

401  HNLPYNYASF SKANEMTLRT LRNTALQARD ITKPLPKNLV WEALHTHG                                     448
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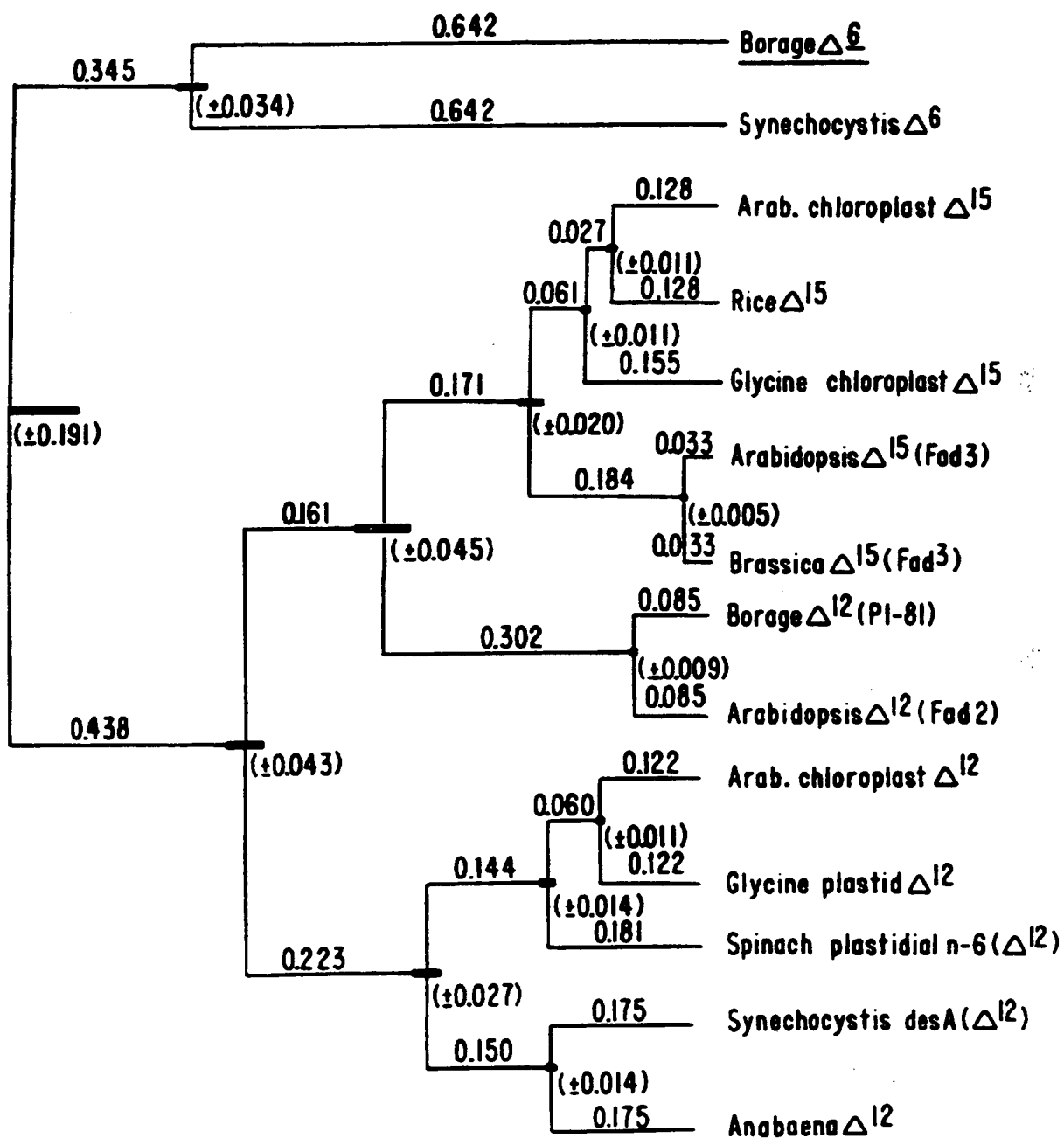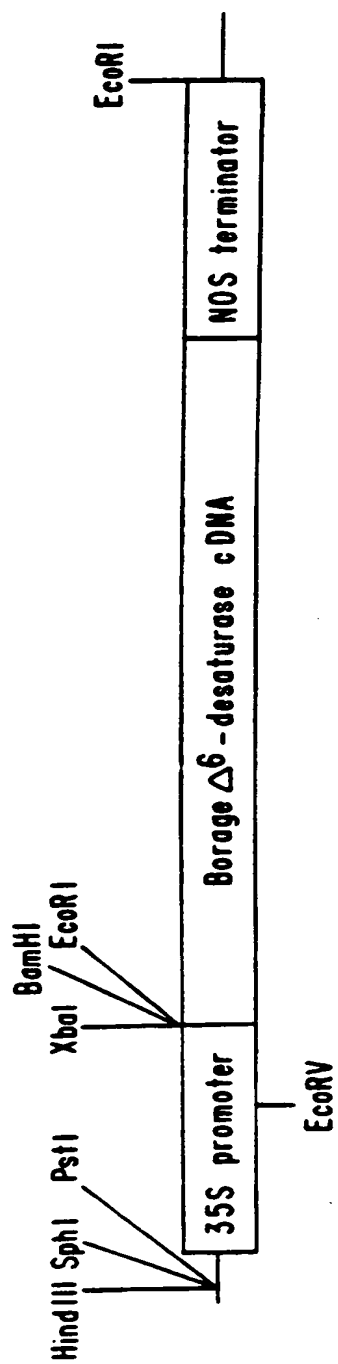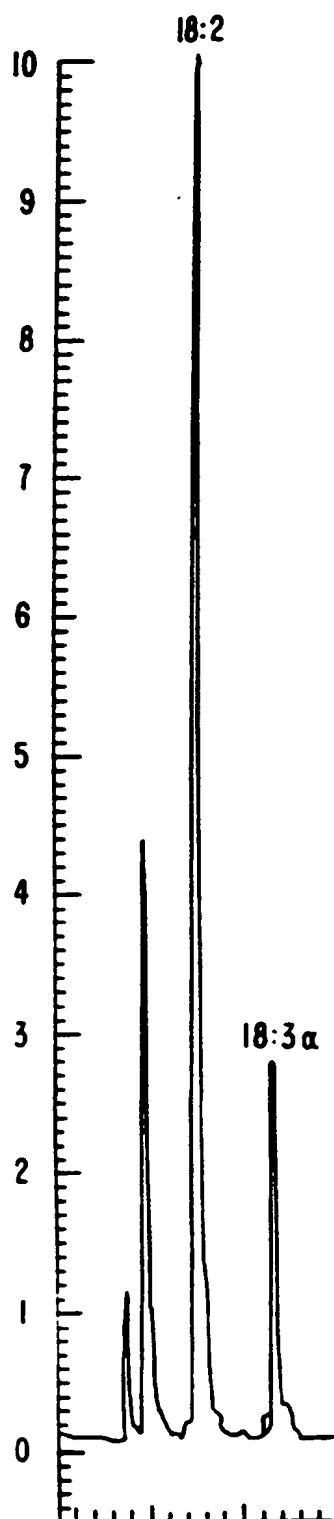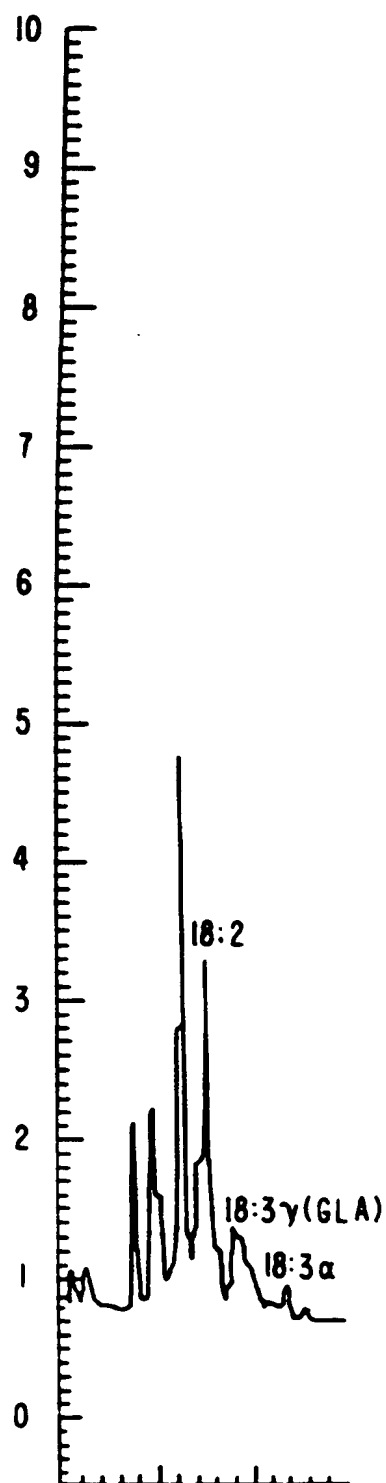
FIG. 6

FIG.7

FIG. 8A

FIG. 8B

FIG.9A
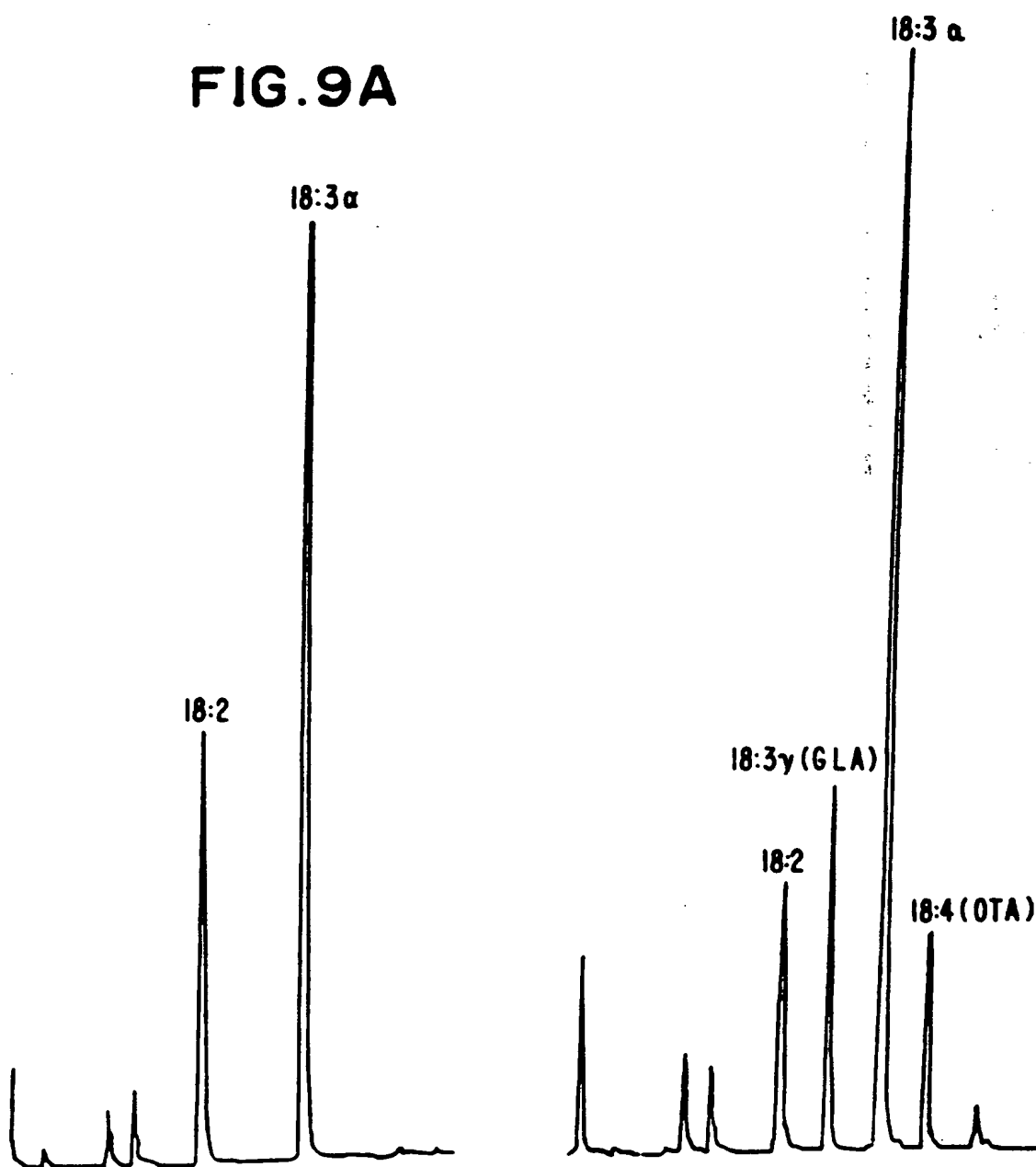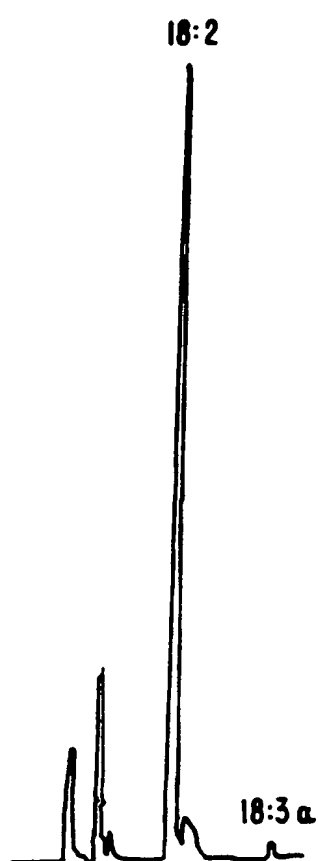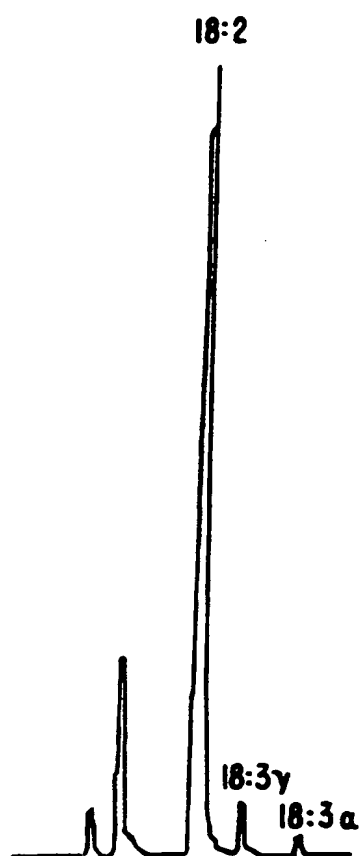
FIG.9B

FIG. 10A

FIG. 10B

PCT

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/53, 15/82, A01H 5/00	A3	(11) International Publication Number: **WO 96/21022**
		(43) International Publication Date: 11 July 1996 (11.07.96)

(21) International Application Number: PCT/IB95/01167

(22) International Filing Date: 28 December 1995 (28.12.95)

(30) Priority Data:
08/366,779   30 December 1994 (30.12.94)   US

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(81) Designated States: AU, BR, CA, CN, JP, RO, RU, UA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report:
12 September 1996 (12.09.96)

(54) Title: PRODUCTION OF GAMMA LINOLENIC ACID BY A Δ6-DESATURASE

(57) Abstract

Linoleic acid is converted into γ-linolenic acid by the enzyme Δ6-desaturase. The present invention is directed to isolated nucleic acids comprising the Δ6-desaturase gene. More particularly, the isolated nucleic acid comprises the promoter, coding region and termination regions of the Δ6-desaturase gene. The present invention provides recombinant constructions comprising the Δ6-desaturase coding region in functional combination with heterologous regulatory sequences. The nucleic acids and recombinant constructions of the instant invention are useful in the production of GLA in transgenic organisms.

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C12N15/53   C12N15/82   A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C12N   A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KADER, J.-C. AND P. MAZLIAK (ED.). PLANT LIPID METABOLISM; 11TH INTERNATIONAL MEETING ON PLANT LIPIDS, PARIS, FRANCE, JUNE 26-JULY 1,1994. XX+588P. KLUWER ACADEMIC PUBLISHERS: DORDRECHT, NETHERLANDS;, NORWELL, MASSACHUSETTS, USA. 0 (0). 1995. 509-511. ISBN: 0-7923-3250-4, XP000569979 GALLE A-M, ET AL.: "Solubilization of DELTA-12- and DELTA-6-desaturases from seeds of borage microsomes." see the whole document	1-28

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
4 July 1996	23.07.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Maddox, A

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PCT/IB 95/01167

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KADER, J.-C. AND P. MAZLIAK (ED.). PLANT LIPID METABOLISM; 11TH INTERNATIONAL MEETING ON PLANT LIPIDS, PARIS, FRANCE, JUNE 26-JULY 1,1994. XX+588P. KLUWER ACADEMIC PUBLISHERS: DORDRECHT, NETHERLANDS;, NORWELL, MASSACHUSETTS, USA. 0 (0). 1995. 21-23. ISBN:0-7923-3250-4, XP000569981 SCHMIDT H, ET AL.: "PCR-based cloning of membrane-bound desaturases" see the whole document ---	1-3
Y	WO,A,93 06712 (RHONE POULENC AGROCHIMIE) 15 April 1993 see the whole document ---	4-28
A	BIOCHEM J 252 (3). 1988. 641-648., XP000568812 GRIFFITHS G: "DELTA-6 AND DELTA-12 DESATURASE ACTIVITIES AND PHOSPHATIDIC ACID FORMATION IN MICROSOMAL PREPARATIONS FROM THE DEVELOPING COTYLEDONS OF COMMON BORAGE BORAGO-OFFICINALIS." see the whole document ---	1-3
A	BIOCHIM BIOPHYS ACTA 1158 (1). 1993. 52-58., XP002007452 GALLE A M, ET AL.: "BIOSYNTHESIS OF GAMMA LINOLENIC ACID IN DEVELOPING SEEDS OF BORAGE BORAGO-OFFICINALIS L. " see the whole document ---	1-3
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
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